Conference Report

59th Tobacco Science Research Conference (TSRC), September 25–28, 2005, Atlanta, Georgia, USA

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This year's 59th Tobacco Science Research Conference (TSRC) held at the Evergreen Conference Center of the Stone Mountain Park was hosted by Schweizer-Mauduit International. In all, more than 330 scientists attended the sessions during which 71 papers and 21 posters were presented.

The Symposium traditionally opened by TSRC was organized this year by Chris Coggins, Carson Watts Consulting, and covered a wide range of topics in keeping with its title "The Tobacco Industry: Changes and Opportunities". In the first paper, Clausen Ely presented the common ground and the differences in the *regulation of tobacco products* between the USA and the EU. Mr Ely is one of the partners of a renowned law firm in Washington DC (Covington & Burling) working in the field of food and drug regulation and has also gained experience in the field of tobacco regulation. According to him, since tobacco was neither a food nor a drug, tobacco products occupied a special position in legislation and/or regulation in the USA as well. There was no special law on tobacco in the USA; sales, marketing as well as information on the composition of cigarette smoke, on nicotine and condensate yields or additives in tobacco products were regulated by a patchwork of laws, voluntary agreements and court orders. Thus, in the absence of any nation-wide, uniform legislation in the USA, many states had imposed local conditions, laws and regulations. There had been attempts and deliberations since 1996 aimed at moving tobacco into the jurisdiction of the Food and Drug Administration (FDA). Ely emphasized that this was not without problems in view of the fact that so far the FDA had no experience with regulating products which did not generate any health benefits.

Jed Rose, Center for Nicotine and Smoking Cessation Research of the Duke University Medical Center in Durham, NC, USA, gave a survey of recent developments in smoking cessation treatment (nicotine inhalation sprays, vaccines, cannabinoid receptor antagonists and partial nicotine receptor agonists).

Lutz Müller from the VdC in Berlin presented the results of an internationally orientated proficiency test in which a defined tobacco sample treated with a known mixture of various common pesticides was quantitatively analyzed for *pesticide residues* by a number of laboratories. The laboratories compared their results with those of the other laboratories anonymously. Two test runs within one year had shown that the considerable differences in the quality of their results had been a stimulus to the laboratories after the first test run, and that they had improved their methods qualitatively and quantitatively within this short period of time.

After this Lutz Müller introduced the pesticide database of the VdC where, as he put it, the measured residues of 191 pesticides of 8 different tobacco types from 80 different countries of origin had been extensively collected. Currently comprising more than 500,000 entries, this database would enable the observation and worldwide management of pesticide use on tobacco on an anonymous basis.

Results obtained so far have shown tobacco smoke to be a low-potency carcinogen in rodents. In the last paper given at the Symposium, Hanspeter Witschi, Center for Health and the Environment of the University of California, Davis, presented the *A/J-mouse as an animal model* for the development of lung tumours. In a study using this simple model, the animals inhaled cigarette smoke over a period of 5 months; and after an interval of 4 months for recovery the tumours on the lung surface were counted. According to Witschi, four different laboratories had definitely shown that inhalation of tobacco smoke had led to an increase in the number of tumours. But weaknesses still remained in this relatively simple model. Lung tumours in mice differed from those in humans. Thus, 80% of the tumours in A/J-mice were found to be benign and could recede again. Although a small number of adenocarcinomas had also developed in mice, this had occurred independently of the inhalation of tobacco smoke. Another shortcoming of this model was its low sensitivity. Thus, in order to demonstrate lower effects at a statistically significant level too, i.e. a 20–30% reduction in

tumour formation by chemo-preventive agents, a large number of animals would have to be used per study group. Benzo[a]pyrene and tobacco-specific nitrosamines had not been the mainly responsible lung carcinogens in tobacco smoke in A/J-mice, whereas the animals had shown a very strong reaction to 1,3-butadiene.

The following sections of this report will describe only a small selection of the large number of papers and posters presented at the 59th TSRC, rather than giving an account of the whole range of scientific topics dealt with at the Conference.

Standardized methods for machine smoking such as those according to the International Organization for Standardization (ISO) or Federal Trade Commission (FTC) do not reflect *individual smoking behaviour* in man. Thus a smoker's daily exposure in terms of cigarette smoke constituents cannot be determined on the basis of the standard values obtained by smoking machines. In his presentation, Michael Borgerding, R.J. Reynolds Tobacco Company, made a comparison between the condensate values determined using five different machine smoking methods (FTC, "Massachusetts", "Canadien Intense", as well as two intense smoking methods without filter blocking) with filter analyses of cigarettes smoked by test subjects. In this context, Borgerding referred to the data from filter analyses by St. Charles, taking into consideration the analyses of a total of 22000 smoked cigarette filters of 28 different brands smoked by 1000 test persons. As a result, 1161 daily average smoke yields were obtained. If the 1981 Report of the Surgeon General were taken into account, recommending a method which produces maximum values for uptake in smokers, it would be found that the machine smoking condition at a puff volume of 45 mL, a puff duration of 2 s and a puff interval of 40 s with non-blocked filter ventilation holes produces values which for most brands and smokers lie clearly within the highest range of the uptake values. The "Canadien Intense" method, in contrast, which had been developed with the aim of approximating real smoking conditions in human smokers would lead to unrealistically high uptake values in smokers.

Using different parameters for machine smoking of cigarettes led to changes in condensate values and/or to quantitative differences in smoke composition. However, up to now relatively little was known about qualitative changes in smoke composition due to changes in smoking conditions. Jianxin Yu, R.J. Reynolds Tobacco Company, presented data on the influence of smoking conditions on nicotine content in smoke. As he pointed out, more intensive smoking conditions had led to a decrease in nicotine content in mainstream smoke, favouring its content in sidestream smoke, since pyrolysis and oxidation of nicotine increased in mainstream smoke whereas they decreased in sidestream smoke. While in sidestream smoke only small amounts of nicotine had been found in the gas phase, nicotine had been detected in mainstream smoke only in the particulate phase at all smoking parameters.

Another topic discussed at the TSRC was the development of Potentially Reduced Exposure Products, *PREPs*. These are modified tobacco products which reduce the exposure of the smoker to toxins in tobacco smoke.

In four presentations, John H. Lauterbach, Lauterbach & Associates, illustrated the difficulties involved in develop-

ing PREPs. The basic idea in developing PREPs was that reduced exposure should lead to a reduced health risk. Up to now, however, it had not become clear which specific components (PAHs, the gas phase, radicals, aromatic amines etc.) needed to be reduced to what extent in order to reduce the health risk too. Lauterbach commented critically on the experiments carried out to determine the most dangerous substances in cigarette smoke via the inhalation risk factors of single substances. In spite of this, the first requirement for a product of this type would seem to be of chemico-analytical nature. In this context the strategy of reducing condensate and nicotine levels generally by means of ventilation and filtration, thus producing "light" and "ultra-light" cigarettes, had not been as successful as desired, since smokers changed their behaviour to compensate for the lower uptake of tobacco smoke and nicotine. Indicators for reduced exposure and for a reduced risk should be measurable in biomarker studies, for instance by reduced cotinine levels or the NNAL or NNAL-glucoside content in urine.

Clausen Ely emphasized during the symposium that any future regulation of tobacco products and the conditions which PREPs would have to meet within such regulations would present a challenge for everyone involved, which would in addition have a significant influence on the development of new tobacco products. The FDA would have to certify the overall beneficial effects for the health of the population accruing from the development of products which could constitute a reduced risk to the consumer when granting approvals for them, which could, depending on the circumstances, turn out to be an insurmountable hurdle. The wording of the EU Directive 2001/37/EG and formulations in texts from the WHO Framework Convention on Tobacco Control (FCTC) concerning how PREPs could be recognized as such and regulated, were full of ambiguity and revealed uncertainty.

A total of five papers given by Simon Yee, Lorillard Tobacco Company, were filled with findings about the pro-inflammatory response triggered *in vitro* in various human cell lines [A549 cells (human alveolar lung cells), NHBE cells (normal human bronchial epithelial cells) and U937 cells (human lymphoma cells with characteristics of monocytes)], by incubation with cigarette smoke condensate (CSC). All three cell lines had responded after an incubation period of 18 hours with CSC by producing the pro-inflammatory cytokines interleukin-6 (IL-6) and interleukin-8 (IL-8) in relation to the dose. This reaction was effectively observed after 18 hours, but not after incubation with CSC for 3 hours. In A549 cells, the gas phase had also led to the production of IL-6 and IL-8. The response to condensate and to the gas phase was of similar intensity. An important early messenger, tumour necrosis factor alpha (TNF- α), could not be detected in the medium. Simultaneous incubation with condensate and antibodies against TNF- α in A549 cells, however, led to a significant decline in the production and release of IL-6 and IL-8 into the medium. This effect was only observable if the antibodies were administered in the first three hours. If the antibodies were added later, the production of interleukin would not be inhibited. The working group interpreted these findings as meaning that TNF- α played a role in this process, but was bound relatively quickly by TNF- α receptors and could

therefore not be detected in the medium. Neither incubation with nicotine nor the tobacco-specific nitrosamine NNK by themselves had stimulated the production or release of IL-8. Additional nicotine, in contrast, had inhibited the release of IL-8 by CSC and increased the production of prostaglandin-2.

The experiments demonstrated that mediation of the toxic effects of cigarette smoke condensate *in vitro* is receptor-controlled as well. Thus the experiments with nicotine suggest a possible involvement of nicotinic acetylcholine receptors (nACh-R), which are likewise expressed in tissue cells of the respiratory tract (1, 2). It would make sense to characterize cell lines which might possibly be used for *in vitro* toxicity tests in terms of the expression, or the change in the expression of nACh receptors.

Scientists from Health Canada presented a poster with data which showed a connection between the proportion of *heterocyclic amines* (HA) in tobacco smoke and the mutagenicity of the tobacco smoke in the Ames test. The content of six different HAs was first measured in the mainstream smoke of ten different Canadian cigarette brands. The specific mutagenicity of the condensate from these cigarettes in the Ames test was correlated with the HA content measured. It proved possible to establish a positive relationship between the content of HAs in the mainstream smoke and the specific mutagenicity of the condensate in question. The strongest correlation was observed between the HA content of the smoke and the number of revertants in TA98. This led the authors to conjecture that heterocyclic amines trigger frame shift mutations rather than base pair substitutions. A control using corresponding amounts of pure substances during the experiments would have thrown more light on these effects.

The Canadian cigarette industry has been collaborating with tobacco growers since 2001 to reduce the content of *tobacco-specific nitrosamines* in tobacco by means of modifications in the curing process. Direct curing over an open fire has been replaced by indirect heating processes. Health Canada presented the measured data of the major nitrosamines (NNN, NNK, NAT and NAB) in tobacco as well as in the mainstream and sidestream smoke of nine popular brands of Canadian cigarettes. The changes in the curing process had led over the last five years to a considerable reduction in NNK (67%), NNN (56%), NAT (65%) and NAB (100%) in tobacco. In general, only a small part of these nitrosamines was transferred into cigarette smoke. Thus in the cigarette brands investigated, the mean content of these nitrosamines was about 80 ng/cig in mainstream smoke (2000: ca. 186 ng/cig) and some 125 ng/cig in sidestream smoke (2000: ca. 180 ng/cig) with condensate and nicotine levels remaining unchanged (all values according to ISO). It would have been another interesting

approach to investigate whether these changes in the tobacco and in tobacco smoke were also reflected in the biological response (as with the data on heterocyclic amines presented).

Cigarette smoke condensate is quite clearly mutagenic at a dose-related level in the *bacterial mutagenicity test* developed by Ames and his co-workers in the Seventies. But any test system should be reproducible and capable of making a quantitative distinction between cigarettes which may differ only slightly in the condensate and nicotine levels measured using smoking machines, but have a different composition. K.W. Fowler, R.J. Reynolds Tobacco Company, showed that the mutagenicity of a test cigarette would need to diverge from a reference cigarette by ca. 20–24% in order to make a statistically significant difference visible if the test is only repeated once. This finding was confirmed by the results of a poster presented by Raymond Proudlock (Charles River Laboratories, Montreal, Canada, in cooperation with Imperial Tobacco Canada).

There are currently no guidelines and standards for toxicological testing of tobacco products (particularly: additives). Jürgen Hahn (Chemisches und Veterinäruntersuchungsamt, Sigmaringen, Germany), member of the DIN Working Group “Toxicological Evaluation of Additives”, set up by the DIN Working Committee “Analysis of Tobacco and Tobacco Smoke”, presented the DIN Technical Report 133 in summarized form on a poster. This report is to form the basis for developing the future regulatory standards in Germany and is intended to help in collecting and assessing toxicological data on additives in tobacco products.

The plant physiologist Dr. T.C. Tso received the “Lifetime Achievement Award” of the TSRC this year in honour of his scientific life’s work with more than 230 publications in the field of applied tobacco research.

The 60th TSRC will take place next year in Montreal, Canada.

REFERENCES

1. Maus, A.D., E.F. Pereira, P.I. Karachunski, R.M. Horton, D. Navaneetham, K. Macklin, W.S. Cortes, E.X. Albuquerque, and B.M. Conti-Fine: Human and rodent bronchial epithelial cells express functional nicotinic acetylcholine receptors; *Mol. Pharmacol.* 54 (1998) 779–788.
2. Zia, S., A. Ndoye, V.T. Nguyen, and S.A. Grando: Nicotine enhances expression of the alpha 3, alpha 4, alpha 5, and alpha 7 nicotinic receptors modulating calcium metabolism and regulating adhesion and motility of respiratory epithelial cells; *Res. Commun. Mol. Pathol. Pharmacol.* 97 (1997) 243–262.