Reduced exposure evaluation of an Electrically Heated Cigarette Smoking System. Part 1: Non-clinical and clinical insights

Matthias K. Schorp*, Anthony R. Tricker, Ruth Dempsey

Philip Morris International R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, 2000 Neuchâtel, Switzerland

Abstract

The following series of papers presents an extensive assessment of the Electrically Heated Cigarette Smoking System EHCSS series-K cigarette vs. conventional lit-end cigarettes (CC) as an example for an extended testing strategy for evaluation of reduced exposure. The EHCSS produces smoke through electrical heating of tobacco. The EHCSS series-K heater was designed for exclusive use with EHCSS cigarettes, and cannot be used to smoke (CC). Compared to the University of Kentucky Reference Research cigarette 2R4F and a series of commercial CC, mainstream cigarette smoke of both the non-menthol and menthol-flavored EHCSS cigarettes showed a reduced delivery of a series of selected harmful and potentially harmful constituents (HPHC), mutagenic activity determined using the Salmonella typhimurium Reverse Mutation (Ames) assay, and cytotoxicity in the Neutral Red Uptake Assay. Clinical evaluations confirmed reduced exposure to HPHC and excretion of mutagenic material under controlled clinical conditions. Reductions in HPHC exposure were confirmed in a real-world ambulatory clinical study. Potential biomarkers of cardiovascular risk were also reduced under real-world ambulatory conditions. A modeling approach, ‘nicotine bridging’, was developed based on the determination of nicotine exposure in clinical evaluations which indicated that exposure to HPHC for which biomarkers of exposure do not exist would also be reduced.

© 2012 Elsevier Inc. Open access under CC BY-NC-ND license

1. Introduction

There is an overwhelming medical and scientific consensus that cigarette smoking is causally related to lung cancer, heart disease, emphysema, and other serious diseases in smokers (US Department of Health and Human Services, 2010). There is no ‘safe’ cigarette and the best way for smokers to reduce the adverse health consequences of smoking is to quit.

For many years the public health communities’ primary goal with respect to tobacco control has focused on reducing initiation, encouraging smoking cessation, and preventing relapse. There has been a growing interest in recent years, however, in alternative approaches including that of harm reduction (Gori, 1980; Institute of Medicine, 2001, 2012; Rodu and Godshall, 2006; Sweanor et al., 2007; Hatsukami et al., 2007; World Health Organization, 2007; Royal College of Physicians and Surgeons, 2007; Gilmore et al., 2009; Zeller et al., 2009; Family Smoking Prevention and Tobacco Control Act, 2009), stimulated perhaps by the observations that in spite of the significant efforts directed towards tobacco control and communication of the risks of smoking, many smokers still have little interest and/or success in quitting smoking. For example, according to the Surgeon General, although about 45% of smokers quit for a day, only approximately 5% succeed in obtaining long-term abstinence (US Department of Health and Human Services, 2010). The World Health Organization (WHO) Study Group on Tobacco Product Regulation has defined tobacco harm reduction as ‘minimizing harms and decreasing total morbidity and mortality, without completely eliminating tobacco and nicotine use’ (World Health Organization, 2007).

Amongst the literature surrounding the questions of harm-reduced products, much of the focus is on the requirements of an effective risk evaluation system. A significant development in tobacco control in the US has been the enactment of the Family Smoking Prevention and Tobacco Control Act (FSPTCA) (Family Smoking Prevention and Tobacco Control Act, 2009), which empowers the US Food and Drug Administration (FDA) to evaluate and regulate Modified Risk Tobacco Products (MRTPs) (Deyton et al., 2010). The FSPTCA defines a MRTP as ‘any tobacco product that is sold or distributed for use to reduce harm or the risk of tobacco-related disease associated with commercially marketed tobacco products.’ The FDA has also been charged to issue guidance or regulations on the scientific evidence required for the assessment and ongoing review of MRTPs in consultation with the US...
Institute of Medicine (IOM), and published a Draft Guidance on “Modified risk Tobacco Product Applications” in March 2012 (Food and Drug Administration, 2012a).

The FSPTCA provides for the approval of an MRTP when reduced exposure or reduced risk has been demonstrated. Different levels of evidence are required for these respective approvals, with correspondingly greater ability for communicating product attributes. The FSPTCA requires applicants to demonstrate that the product, as actually used, will: (i) significantly reduce harm and the risk of tobacco-related disease to individual tobacco users; and (ii) benefit the health of the population as a whole, taking into account both users of tobacco products and persons who do not currently use tobacco products. The FSPTCA’s recognition that harm reduction now has a statutory place alongside the regulations of food and medicine provides the platform for moving forward and a source of confidence that effective, appropriate MRTPs can be developed and commercialized.

The studies presented in this series of papers were performed prior to the enactment of the FSPTCA, and publication of the IOM Report (Institute of Medicine, 2012). At the time, we focused on evaluating exposure reduction at ‘three levels’: firstly at the ‘product level’ (i.e., does the product have a reduced yield of a HPHC under a variety of laboratory conditions), secondly at the ‘individual smoker level’ (i.e., do smokers using these products experience reductions in their exposure to specific HPHC), and finally at the ‘population level’ (i.e., is this exposure reduction likely to be realized by both a significant proportion of the normal smoking population given that they are likely to represent a wide range of ‘actual use’ smoking behaviors). Three considerations appeared to be essential. Firstly, the product characterization, as determined in laboratory studies, should not be limited to comparisons under standardized smoking conditions but emulate anticipated conditions of actual use. Secondly, uptake of relevant HPHC should be determined in populations that are representative of those who are most likely to use the product (Hatsukami et al., 2007, 2012; World Health Organization, 2007). The latter requires valid biomarkers of exposure as well as selection of appropriate populations and reference products that can be considered as reasonably representative of those used by smokers who may switch to using the new products. Thirdly, consideration of the potential reduction in exposure by non-smokers to environmental aerosols produced by the MRTP vs. environmental tobacco smoke (ETS) from CC is also needed. Tricker et al. (2009) has published a comparative indoor air quality assessment of EHCSS series-K vs. a CC.

Clearly, in consideration of both the Draft Guidance on “Modified Risk Tobacco Product Applications” (Food and Drug Administration, 2012a) and the IOM Report (Institute of Medicine, 2012), further work is needed in order to meet such a standards. We nevertheless consider that product testing is an iterative process and the data reported here should be considered as relevant, although not sufficient, for the evaluation of reduced exposure, reduced risk, and population harm.

Although the causal relationship between smoking and several diseases has been well established (Doll et al., 2004), there is still very little understanding of the underlying mechanisms. More than 5300 chemical compounds have been identified in cigarette tobacco smoke (Rodgman and Perfetti, 2009). Public health authorities and representatives now propose some 100 HPHC as possible causes of smoking-related diseases such as lung cancer, heart disease, and emphysema (Health Canada 2000; Food and Drug Administration, 2012b; Talhout et al., 2011). There is no consensus, however, that lowering or eliminating any single compound (or even a combination of compounds) in smoke would have a significant impact on risk. Partly in response to this dilemma, the IOM introduced the concept of a ‘Potential Reduced-Exposure Product’ (PREP) (Institute of Medicine, 2001), based on a first assumption that reduction of exposure is related to a reduction in harm.

We have focused on the development of products that substantially reduce or eliminate a wide spectrum of HPHC. Our current approach achieves this by eliminating direct tobacco combustion and limiting tobacco pyrolysis by heating at significantly lower temperatures than encountered in CC. However, the IOM and others conclude that simply reducing exposure does not necessarily equate to harm reduction (Institute of Medicine, 2001; World Health Organization, 2007; Zeller et al., 2009). Thus, a comprehensive assessment of reduced exposure is necessary, but is not sufficient for determining a modified tobacco product’s potential to reduce risk. Novel testing strategies have been recently proposed by the IOM (Institute of Medicine, 2012).

The following series of papers presents an extensive assessment of the EHCSS series-K cigarette vs. CC as an example for an extended testing strategy for evaluation of reduced exposure. The concept of reduced exposure in this testing strategy considers a broad range of potential smoking behaviors, and characterizes the potential reductions in exposure to a range of HPHC in cigarette smoke which could be considered to be of importance in relation to smoking-related diseases.

2. The Electrically Heated Cigarette Smoking System (EHCSS)

Tobacco smoke from CC consists of an aerosol containing liquid droplets (‘particulate phase’) suspended in the gas–vapor phase. It is generated by complex and overlapping burning-, pyrolysis-, pyrosynthesis-, distillation-, sublimation-, and condensation processes (Borgerding and Klus, 2005). With minor exceptions, both pyrogenesis and pyrosynthesis of HPHC result from the thermal decomposition from organic tobacco compounds taking place at elevated temperatures (Baker, 2006; Borgerding et al., 1997; Torikai et al., 2005), thus, a reduction of these toxicants may be achieved by generating a simpler smoke aerosol, e.g., by heating rather than burning tobacco (e.g., ECLIPSE Expert Panel, 2000).

The first-generation of the EHCSS (series-E) has been subject to extensive analytical and toxicological evaluation (Patskan and Reinighaus, 2003) demonstrating simplified smoke chemistry compared to the University of Kentucky 1R4F reference research cigarette (Stabbert et al., 2003) and against a series of CC from the US (Roemer et al., 2004). The 1R4F cigarette is considered to be representative of the low ‘tar’ segment of the US cigarette market (Diana and Vaught, 1990). Notable was the significant reduction in carbon monoxide (CO) and increased yield of formaldehyde in EHCSS-E mainstream smoke, compared to the 1R4F cigarette. On a per milligram total particulate matter (TPM) basis the concentration of formaldehyde was increased approximately tenfold (Stabbert et al., 2003). The in vitro genotoxicity and cytotoxicity of mainstream smoke (Tewes et al., 2003; Roemer et al., 2004; Schramke et al., 2006) and the biological activity of mainstream smoke was reduced in a 90-day sub-chronic rat inhalation study, compared to the 1R4F cigarette (Tepstra et al., 2003). A clinical evaluation performed in the US confirmed that exposure to selected mainstream cigarette smoke constituents was reduced (Roethig et al., 2005).

A second-generation EHCSS (series-JLI) was developed in which ammonium magnesium phosphate (AMP) was used in the cigarette paper to replace calcium carbonate (Fourrier and Paine, 2001). It was anticipated that ammonia released during the pyrolysis of AMP would condense with formaldehyde to form hexamethyleneetetramine (HMT). Chemical analysis of smoke from the EHCSS-JLI cigarettes containing AMP showed lower yields of formaldehyde and several reported HPHC, a further decrease in CO yield, and increased yields of ammonia and HMT (Roemer et al., 2004).
The impact of AMP on smoke composition, in vitro cytotoxicity and genotoxicity has been reported in detail (Roemer et al., 2008). Reduced toxicological activity of mainstream smoke was also determined in both a 90-day sub-chronic rat inhalation study and a 35-day study focusing on lung inflammation in rats (Moennikes et al., 2008). Clinical evaluations also confirmed reduced exposure to selected HPHC and reduced excretion of mutagenic material in urine (Roethig et al., 2007, 2008). Further clinical evaluations concluded that switching from CC to the second-generation EHCSS-JLI cigarette improved prognostic markers for cardiac disease assessed by symptom-limited spiroergometry (Unverdorben et al., 2007), heart rate and rate-pressure-product parameters (Unverdorben et al., 2008) after three days of product switching.

The third-generation EHCSS (series-K) electrical heater, which can be used with EHCSS menthol or non-menthol cigarettes provides up to 8 puffs per cigarette (Werley et al., 2008). The EHCSS uses controlled heating of tobacco at a temperature significantly less than encountered in the burning cone of a CC, and CC fail to activate the electronic system incorporated in the puff-activated heater. The EHCSS series-K cigarette contains a column of cigarette tobacco filler, wrapped in a tobacco mat with a cigarette paper overwrap. EHCSS-K3 and EHCSS-K6 cigarettes differ in the construction of the filter, with a more efficient filter being used in the EHCSS-K3 cigarette (Fig. 1).

The series-K cigarette is characterized by a reduced delivery of HPHC in mainstream smoke and reductions in several toxicological endpoints as observed in a battery of in vitro and in vivo assays (Werley et al., 2008). In addition, virtually eliminating the formation of sidestream smoke, which is normally formed by the smouldering of a CC, results in significantly lower concentrations of ETS when EHCSS cigarettes are smoked compared to a CC (Frost-Pineda et al., 2008a; Tricker et al., 2009). Selected biomarkers of exposure to HPHC have been shown to be reduced in clinical evaluations of CC smokers who switched to use the EHCSS-K6 cigarette (Frost-Pineda et al., 2008b,c). Favorable changes towards increased heart rate variability (Munjal et al., 2009) and pulmonary function (Unverdorben et al., 2010) have also been observed after switching from smoking CC to the EHCSS-K6 cigarette for three days.

### 3. Testing strategy

The current strategy is based on both non-clinical and clinical evaluations in which reduced exposure assessment is considered in a translational approach from ‘product level – to smoker level – to population level’. The presented strategy is an extension of previous reduced exposure assessments of the 5 mg ISO tar EHCSS-K6 cigarette (Werley et al., 2008; Frost-Pineda et al., 2008b,c).

A key component of this strategy is the consideration of a range of machine smoking conditions for the laboratory assessments. It is known that smoking topography, e.g., puff volume, puff duration, inter-puff interval, varies greatly among smokers (Schorp, 2005), and this may explain, in part, the significant within- and between-smoker variability of nicotine uptake and toxicant exposure (Byrd et al., 1998; Jarvis et al., 2001; Ueda et al., 2002; Scherer et al., 2007a; Fidler et al., 2008; Mendes et al., 2009; Lindner et al., 2011). Consequently, we have investigated the performance of the products under 25 different machine smoking conditions reflective of multiple human smoking topographies. These laboratory studies include extensive smoke chemistry analysis in addition to in vitro assessments.

In addition, we have selected the CC used as comparator/reference products in the studies (Table 1) based on our understanding of the type of CC smoked by the populations considered most likely to switch to the EHCSS series-K cigarette in a number of different countries. It was considered essential, for example, to ensure that any reduction in exposure that may be achieved by switching to the EHCSS would remain valid when compared to exposure resulting from using a representative CC with low International Organization for Standardization (ISO) tar and nicotine yields. With these considerations in mind, six different CC were selected as benchmarks that either matched the ISO tar delivery of the EHCSS series-K cigarettes or represented the lowest ISO tar delivery of commercially available cigarettes in the countries in which clinical evaluations were performed (Table 1).

In selecting the sites for the clinical studies, we chose countries for which we had reason to believe smoking behavior patterns might be quite different. There is, for example, a general under-
standing that smokers in Japan have different smoking behaviors and taste preferences for mentholated products compared to smokers in Western Europe (Ueda et al., 2002; Giovino et al., 2004) while Korea represents a cigarette market in which smokers have a preference for smoking cigarettes with very low smoking machine-measured ISO tar and nicotine yields.

4. In vitro toxicological assessment of test and marketed reference cigarettes

In Part 2 of this series of papers (Zenzen et al., 2012), ‘product level’ testing was performed to determine up to 49 HPHC in mainstream smoke of EHCSS-K3, EHCSS-K6, EHCSS-K6M and four representative CC (M6UK, PM1, M6J, Lark1) according to ISO machine smoking conditions (International Organization for Standardization, 2000). The list of HPHC determined included compounds recommended by the US Consumer Product Safety Commission (US Consumer Products safety Commission in Consultation with the US Department of Health and Human Services, 1993) and evaluated for carcinogenicity (International Agency for Research on Cancer, 1987). The list of compounds analyzed included the determination of all nine HPHC recommended for mandated lowering of exposure levels (World Health Organization, 2008). In addition, smoke chemistry and in vitro toxicological assessment was performed using 25 different machine-smoking regimens delivering a range of nicotine yields between the 10th–90th percentiles of clinically determined nicotine uptake distributions (‘Human Puffing Behavior’ [HPB] regimens). The HPB protocols for each of the four CC were determined using a modeling approach (Urban et al., 2008), and a matrix approach was applied for the EHCSS series-K cigarettes (Zenzen et al., 2012). A subset of the data set (EHCSS-K6, M6UK, and PM1 cigarettes; ISO regimen and 15 additional experimental machine-smoking regimens reflecting HPB) was used to develop the ‘nicotine bridging’ method (Urban et al., 2012). The HPB regimens were used since standard machine-smoking protocols are not representative of human smoking behavior and cannot be used to predict the actual exposure of a smoker (Gori and Lynch, 1985).

In vitro toxicological assessment was performed to assess bacterial mutagenicity of the smoke particulate phase (condensate) towards three tester strains of Salmonella typhimurium (TA98, TA100, and TA1537 with S9 activation) in the Salmonella reverse mutation assay (Maron and Ames, 1983) according to recommendations by the Organization for Economic Co-operation and Development (Organization for Economic Co-operation and Development, 1997) and International Conference on Harmonization (International Conference on Harmonization, 1995). These strains were not used to determine excretion of mutagenic material in the urine of smokers in clinical studies (Tricker et al., 2012a,b,c,d). Instead, the strain YG1024, an O-acetyltransferase-overproducing derivative of TA98, was used which is more sensitive to the presence of mutagens in urine (Einstöp et al., 1990; De Flora et al., 1995; Kuenemann-Migeot et al., 1997).

Cytotoxicity of both the particulate and the gas–vapor phase of mainstream smoke were determined by the Neutral Red Uptake (NRU) assay according to INVITTOX protocol No. 3a (INVITTOX, 1990). The test material was generated using both ISO and HPB machine-smoking regimens.

These non-clinical evaluations served to address four main objectives:

- To understand the new product’s potential to reduce exposure based on reductions in smoke chemistry as compared to CC using multiple smoking regimen,
- To provide quantitative data to design clinical studies to test reductions in exposure to selected HPHC in the new product,
- To assess acceptability of the new product for use in human clinical studies, the minimum criteria of which was to ensure that the product would not present an increased or new hazard in comparison to CC, and
- To provide a broad range of measures to characterize the product which could not be directly determined in clinical evaluations.

5. Clinical evaluations

Controlled clinical studies are reported in Parts 3–7 of this series of papers (Martin Leroy et al., 2012; Tricker et al., 2012a,b,c,d). Studies were performed to determine the ‘smoker level’ exposure to selected HPHC when using test (i.e., EHCSS) and reference (i.e., CC) products. In order to substantiate the potential of a new tobacco product to reduce the exposure to HPHC, a reliable panel of biomarkers for assessing exposure in human smokers was used (World Health Organization, 2008). The panel of biomarkers of exposure to selected HPHC was selected based on (i) previously determined smoke chemistry (Part 2; Zenzen et al., 2012), (ii) ability of the biomarker of exposure to determine differences in exposure of the parent compound in cigarette smoke (Hecht, 2003; Feng et al., 2006; Carmella et al., 2009; Scherer et al., 2007b), and (iii) validation of the analytical methods for the determination of the biomarker in urine according to US FDA guidance (Food and Drug Administration, 2001). Individual tobacco smoke-specific and tobacco smoke-associated biomarkers of exposure were also selected depending on the individual study protocols resulting in a panel of biomarkers for the assessment of exposure to 12 selected HPHC and excretion of mutagenic material in urine (Table 2).

The panel of biomarkers of exposure included five of the nine toxicants (1,3-butadiene, acrolein, benzene, carbon monoxide, and 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanone [NNK]) recommended for mandated lowering in cigarette mainstream smoke (World Health Organization, 2008). Of the remaining four smoke toxicants (acetaldehyde, benzo(a)pyrene, formaldehyde, and N-nitrosornicotine), suitable biomarkers of exposure and/or analytical methods were not available at the time of the studies. The panel of biomarkers of exposure included:

- Nicotine and its metabolites since these are well established tobacco-specific biomarkers for assessment of exposure to cigarette smoke (Society for Research on Nicotine and Tobacco Subcommittee on Biochemical Verification, 2002; Tricker, 2006). On a quantitative basis, the determination of the concentration of the molar sum of nicotine, cotinine, trans-3’-hydroxycotinine, and their respective glucuronide conjugates, expressed as nicotine and its metabolites were determined in conformity with International Organization for Standardization (ISO) methods. Puff count was set to 8 puffs based on lighter design, and data were obtained when the EHCSS-K was smoked on a linear smoking machine.

Table 1

<table>
<thead>
<tr>
<th>Cigarette</th>
<th>Brand name</th>
<th>Tar [mg/cig.]</th>
<th>Nicotine [mg/cig.]</th>
<th>CO [mg/cig.]</th>
</tr>
</thead>
<tbody>
<tr>
<td>EHCSS-K3</td>
<td>–</td>
<td>3</td>
<td>0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>EHCSS-K6</td>
<td>–</td>
<td>5</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>EHCSS-K6M</td>
<td>–</td>
<td>5</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>M6UK</td>
<td>Marlboro</td>
<td>6</td>
<td>0.5</td>
<td>7</td>
</tr>
<tr>
<td>M6J</td>
<td>Marlboro</td>
<td>6</td>
<td>0.5</td>
<td>7</td>
</tr>
<tr>
<td>M6JMQ</td>
<td>Marlboro Ultra Lights Menthol</td>
<td>4</td>
<td>0.3</td>
<td>5</td>
</tr>
<tr>
<td>PM1</td>
<td>Philip Morris One</td>
<td>1</td>
<td>0.2</td>
<td>2</td>
</tr>
<tr>
<td>Lark1</td>
<td>Lark One</td>
<td>1</td>
<td>0.1</td>
<td>2</td>
</tr>
<tr>
<td>Lark1MQ</td>
<td>Lark One Menthol</td>
<td>1</td>
<td>0.1</td>
<td>2</td>
</tr>
</tbody>
</table>
Table 2
Summary of smoke constituent and biomarkers of exposure determined in the EHCSS clinical evaluations.

<table>
<thead>
<tr>
<th>Smoke constituent</th>
<th>Biomarker of exposure</th>
<th>Country of evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,3-Butadiene</td>
<td>Monohydroxybutenyl mercapturic acid (MHBMA)</td>
<td>Tricker et al. (2012a)</td>
</tr>
<tr>
<td>2-Naphthyamine</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4-Aminobiphenyl</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Acrolein</td>
<td>3-Hydroxypropyl mercapturic acid (3-HPMMA)</td>
<td>–</td>
</tr>
<tr>
<td>Acrylamide</td>
<td>Acrylamide mercapturic acid (AAMA)</td>
<td>–</td>
</tr>
<tr>
<td>Benzene</td>
<td>5-Phenyl mercapturic acid (5-PMMA)</td>
<td>–</td>
</tr>
<tr>
<td>Carbon monoxide</td>
<td>Carbon monoxide (CO)</td>
<td>–</td>
</tr>
<tr>
<td>Crotonaldehyde</td>
<td>3-Hydroxy-1-methylpropyl mercapturic acid (3-HMPMA)</td>
<td>–</td>
</tr>
<tr>
<td>Nicotine</td>
<td>Cotinine (COT-P)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Nicotine equivalents (NEq)</td>
<td>–</td>
</tr>
<tr>
<td>NNKa</td>
<td>Total 4-(methylamino)-1-(3-pyridyl)-1-butanol (NNAL)c</td>
<td>–</td>
</tr>
<tr>
<td>Pyrene</td>
<td>Total 1-hydroxypyrene (1-OHP)d</td>
<td>–</td>
</tr>
<tr>
<td>o-Toluidine</td>
<td>o-Toluidine (o-TOL)</td>
<td>–</td>
</tr>
<tr>
<td>Mutagens</td>
<td>Salmonella mutagenicity (YG1024 with S9)</td>
<td>–</td>
</tr>
</tbody>
</table>

**a** NNK, 4-(methylamino)-1-(3-pyridyl)-1-butane.

**b** Nicotine equivalents (NEq) were determined as the molar sum of nicotine, cotinine, and trans-3'-hydroxycotinine plus their respective glucuronide conjugates.

**c** Total 4-(methylamino)-1-(3-pyridyl)-1-butanol (NNAL) was determined as the molar sum of 4-(methylamino)-1-(3-pyridyl)-1-butanol and its O-glucuronide conjugate.

**d** Total 1-hydroxypyrene (1-OHP) was determined as the molar sum of 1-hydroxypyrene and its glucuronide and sulfate conjugates.

Nicotine equivalents (NEq), in 24-h urine provides an estimate of approximately 85% of the total nicotine uptake [Benowitz et al., 1994; Tricker, 2006]. In addition, serum cotinine and plasma nicotine were also determined in some of the clinical evaluations [Benowitz, 1988].

Carboxyhemoglobin (COHb) was selected as a biomarker of CO exposure based on its classical use for determination of tobacco smoke exposure [Rieben, 1992; Society for Research on Nicotine and Tobacco Subcommittee on Biochemical Verification, 2002; Scherer, 2006].

Total 4-(methylamino)-1-(3-pyridyl)-1-butanol (NNAL) plus its O-glucuronide conjugate 4-[(methylamino)-1-(3-pyridyl)but-1-yl]-O-D-glucosiduronic acid (NNAL-Gluc) was determined as a tobacco-specific biomarker of exposure to NNK [Hecht and Tricker, 1999].

Total 1-hydroxypyrene (1-OHP) plus its glucuronide and sulfate conjugates [Strickland et al., 1996] was determined as a surrogate marker for the total concentration of polycyclic aromatic hydrocarbons (PAHs) present in cigarette smoke [Brandt and Watson, 2003].

2-Naphthyamine (2-NA), 4-aminobiphenyl (4-ABP), and o-toluidine (o-TOL) were determined directly in urine [Riedel et al., 2006] as representative aromatic amines present in cigarette smoke [Matsuda and Hoffmann, 1969; Patrianakos and Hoffmann, 1979].

N-Acetyl-S-(2-carbamoylthyl)-L-cysteine (AAMA) and N-(RS)-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA) were determined in urine as biomarkers of exposure to acrylamide [Urban et al., 2006].

1-Hydroxy-2-(N-acetylcycteinyl)-3-butene and 1-(N-acetylcycteinyl)-2-hydroxy-3-butene (collectively called MHBMA for monohydroxybutenyl mercapturic acid) were determined in urine as a biomarker of exposure to 1,3-butadiene [van Sittert et al., 2000].

3-Hydroxy-1-methylpropyl mercapturic acid (HMPMA) was determined as a biomarker of exposure to crotonaldehyde, an α,β-unsaturated aldehyde present in cigarette smoke [Scherer et al., 2007b].

S-Phenyl mercapturic acid (S-PMMA) was selected from several known metabolites of benzene as a biomarker of exposure to benzene in tobacco smoke [Melikian et al., 1993; Fustinoni et al., 2005].

3-Hydroxypropyl mercapturic acid (3-PMMA) was selected as a biomarker of exposure to acrolein [Mascher et al., 2001].

In addition, Salmonella typhimurium YG1024 was used to determine excretion of mutagenic material in urine [Einstött et al., 1990].

The clinical studies had one primary objective: To comparatively assess exposure reductions of EHCSS vs. CC smoke HPHC, when these products were used by different smoking populations. This testing strategy extends the observed differences in smoke chemistry reductions using standardized machine-smoking protocols ('product level'), to a measure of actual uptake in a controlled clinical environment ('smoker level'), minimizing biases such as dual use, or differential exposures from other sources. This approach partially addresses differences in smoking behavior and exposure to tobacco smoke HPHC, albeit with some limitations. For example, the circumstances of use within the clinical environment may be quite artificial and the maximum actual use level of the EHCSS (i.e., number of smoked cigarettes per day) was limited to the determined consumption of CC at Baseline. Thus, subjects could not increase their use of EHCSS above the number of CC they had originally smoked, i.e., one possible method for compensation may be quite artificial and the maximum actual use level of the EHCSS ('smoker level'), minimizing biases such as dual use, or differential exposures from other sources. This approach partially addresses differences in smoking behavior and exposure to tobacco smoke HPHC, albeit with some limitations. For example, the circumstances of use within the clinical environment may be quite artificial and the maximum actual use level of the EHCSS (i.e., number of smoked cigarettes per day) was limited to the determined consumption of CC at Baseline. Thus, subjects could not increase their use of EHCSS above the number of CC they had originally smoked, i.e., one possible method for compensation was, in effect, prohibited by the study design [Scherer, 1999].

In Part 3 of this series of papers, an 8-day randomized, controlled, open-label, parallel-group, single-center study design was used to compare biomarkers of exposure to nine selected HPHC in cigarette smoke (Table 2) in 160 male and female Caucasian subjects smoking the M6UK cigarette at baseline who were randomized to continue smoking M6UK cigarettes, or switch to EHCSS-K3, EHCSS-K6, or PM1 cigarettes (for cigarette definitions see Table 1), or to no-smoking [Tricker et al., 2012a]. The study was conducted in Belfast, Northern Ireland. The primary objectives of the study were to compare exposure to benzene and CO between the study groups on Day 8 vs. baseline (Day 0). The mean decreases from baseline to Day 8 were statistically significant ($p < 0.05$) for
all determined HPHC including CO and benzene, and excretion of mutagenic material in urine in the EHCSS-K3 (range: \(-41.2 \pm 26.6\%\) to \(-83.1 \pm 9.2\%\) [mean ± standard deviation]) and EHCSS-K6 (range: \(-35.5 \pm 29.2\%\) to \(-79.4 \pm 14.6\%\)) groups. The largest reductions in exposure occurred in the no-smoking group (range: \(-55.4 \pm 45.0\%\) to \(-100.0 \pm 0.0\%\)).

In Part 4 of this series of papers, an 8-day randomized, controlled, open-label, parallel-group, single-center study design was used to compare biomarkers of exposure to twelve selected HPHC (Table 2) in urine, in 72 male and female Korean subjects smoking the Lark1 cigarette at baseline who were randomized to continue smoking the Lark1 cigarette, or switch to using EHCSS-K3, or to no-smoking (Tricker et al., 2012b). The study was conducted in Seoul, South Korea. The primary objective of the study was to compare exposure to CO between the study groups on Day 8. CO exposure was significantly lower in the EHCSS-K3 group than in the Lark1 group at Day 8 (p < 0.001). The mean decreases from baseline (Day 0) to Day 8 were statistically significant (all p < 0.05) for 10 of 12 selected HPHC in mainstream cigarette smoke including CO, in the EHCSS-K3 group (range: \(-1.5 \pm 9.9\%\) to \(-74.2 \pm 10.1\%\)). Exposure to acrolein (\(-1.3 \pm 35.8\%\)) was not significantly reduced, and exposure to crotonaldehyde was increased (\(28.1 \pm 155.3\%\)). The largest mean reductions in HPHC occurred in smokers who switched to no-smoking (\(-3.4 \pm 41.8\%\) to \(-98.9 \pm 0.6\%\)). Excretion of mutagenic material in urine was decreased significantly (p < 0.05) in the EHCSS-K3 and no-smoking groups (\(-31.8 \pm 48.8\%\) and \(-45.3 \pm 29.7\%\), respectively).

In Part 5 of this series of papers, an 8-day randomized, controlled, open-label, parallel-group, single-center study design was used to compare biomarkers of exposure to twelve selected HPHC in cigarette smoke (Table 2) in 128 male and female Japanese subjects smoking M6J cigarettes at baseline who were randomized to continue smoking M6J cigarettes, or switch to EHCSS-K3, EHCSS-K6, or Lark1 cigarettes, or to no-smoking (Tricker et al., 2012c). The study was conducted in Osaka, Japan. The primary objective of the study was to compare exposure to CO between the study groups on Day 8. CO exposure was significantly lower in the EHCSS groups than in the Lark1 group at Day 8 (p < 0.001). The mean decreases from baseline (Day 0) to Day 8 were statistically significant (p < 0.05) for all biomarkers of exposure to the selected HPHC including CO, and mutagenic material in urine in the EHCSS-K3 (range: \(-9.8 \pm 60.0\%\) to \(-73.0 \pm 13.0\%\)) and EHCSS-K6 (range: \(-14.6 \pm 51.8\%\) to \(-75.6 \pm 11.4\%\)) groups. The largest reductions in exposure to HPHC (all significant at p < 0.01 level) occurred in the no-smoking group (range: \(-13.7 \pm 90.9\%\) to \(-97.6 \pm 6.5\%\)).

In Part 6 of this series of papers, a 6-day randomized, controlled, open-label, parallel-group, single-center study design was used to compare biomarkers of exposure to twelve selected HPHC in cigarette smoke (Table 2) and serum Clara cell 16-kDa protein, an indicator of lung epithelial injury, in 102 male and female Japanese subjects smoking the M4JM cigarette at baseline who were randomized to continue smoking M4JM cigarettes, or switch to smoking EHCSS-K6M, or switch to Lark1M, or to no-smoking (Tricker et al., 2012d). The study was conducted in Osaka, Japan, and was designed to investigate the effect of menthol in the EHCSS-K6M cigarette. The primary objective of the study was to compare exposure to CO between the study groups on Day 5/6. Exposure to CO was significantly reduced on Days 5/6 for the EHCSS-K6M group than for both M4JM and Lark1M groups (p < 0.001). The mean decreases from baseline (Days \(-1/0\)) to Day 5/6 were statistically significant (p < 0.05) for exposure to CO, most biomarkers of exposure and excretion of mutagenic material in urine in the EHCSS-K6M group (\(-12.3 \pm 34.9\%\) to \(-83.4 \pm 9.7\%\)). The largest mean reductions (p < 0.05) in exposure to CO, most biomarkers of exposure to HPHC and excretion of mutagenic material in urine occurred in the no-smoking group (\(-1.4 \pm 41.0\%\) to \(-93.6 \pm 9.0\%\)). Serum concentrations of Clara cell 16-kDa protein were not significantly changed in all groups, compared to baseline.

In Part 7 of this series of papers, a one-month randomized, open-label, ambulatory, controlled clinical study to compare biomarkers of exposure to ten selected HPHC in cigarette smoke (Table 2) in 316 male and female Polish subjects who smoked their usual brand of CC at baseline and were randomized to either continue smoking their own brand of cigarettes or switch to EHCSS-K6 (Martin Leroy et al., 2012). The study was conducted in Warsaw, Poland. The study was intended to assess whether changes in exposure to HPHC determined in the above short-term clinical confinement studies are representative of reductions in subjects switching to smoke the EHCSS-K6 cigarette under real-life conditions. Biomarker assessments were performed at baseline (Day 0) and at various time points until completion of the study (Day 35). The primary objective of the study was to compare high-sensitivity C-reactive protein (hs-CRP) and white blood cell (WBC) counts after one month (Day 35). Within-group comparisons showed reductions in median serum hs-CRP from baseline (1.37 mg/l) to the end of study (1.11 mg/l) for the EHCSS-K6 study group and from 1.18 to 0.85 mg/l in the CC group. Mean WBC counts decreased from 7.09 \pm 1.73 G/l to 6.90 \pm 1.64 G/l and 7.00 \pm 1.63 G/l to 6.94 \pm 1.60 G/l in the EHCSS-K6 and CC groups, respectively. All biomarkers of exposure to HPHC were decreased in the EHCSS-K6 group at Day 35, although increases in cigarette consumption were observed. However, none of the reductions in biomarkers of exposure between the EHCSS-K6 and CC groups was significant.

6. Nicotine bridging and population level modeling

In Part 8 of this series of papers (Urban et al., 2012), the concept of ‘nicotine bridging’ was used to model additional HPHC uptake distributions based on nicotine uptake distributions obtained for mainstream smoke chemistry analysis of 2 CC and the EHCSS-K6 using the ISO regimen and 15 additional experimental machine-smoking regimens reflecting HPB (Part 2; Zenzen et al., 2012) and a clinical evaluation (Part 3; Tricker et al., 2012a). Modeling HPHC uptake proportional to nicotine uptake distributions serves as a means to assess exposure to HPHC since biomarkers of exposure to nicotine can be directly measured in clinical/population-based studies and nicotine uptake distributions calculated (Urban et al., 2012). It is assumed that exposure distributions for other HPHC for which biomarkers of exposure are not available also show quantitative retention similar to the pulmonary deposition and retention of nicotine, which is almost (i.e., 90–100%) complete (Armitage et al., 2004; Baker and Dixon, 2006). Consequently, differences in exposure to HPHC from different cigarette designs, e.g., in smokers of CC and smokers switching to the EHCSS, can be estimated based on distribution analysis of clinically determined nicotine uptake and smoke chemistry data. Furthermore, reduced exposure assessment can be extended by evaluation of similarity of the CC (‘test’) nicotine uptake distribution in a clinical setting (‘smoker level’) with the population-based nicotine uptake distribution of similar ISO tar yield (‘reference’) cigarettes of the same geographical region (‘population level’). A criterion for similarity (test population/reference population) used was the 90% confidence interval of the median nicotine uptake (ratio of medians of test/reference), which should lie within the interval of 0.8–1.25. This evaluation addresses some concerns related to the applicability of results obtained in a clinical study population to a larger population.
7. Learning's and further elaboration of reduced exposure evaluation

As described in the IOM Report (Institute of Medicine, 2001), population harm (morbidity and mortality associated with tobacco use) is a function of toxicity of the product (per use), the intensity of its use (per user), and the prevalence of use. These product testing components have been further extended by the FSPTCA to include that a MRTP will significantly reduce the risk of tobacco-related disease to individual users, and benefit the health of the population as a whole, taking into account both current and future users of tobacco products (Family Smoking Prevention and Tobacco Control Act, 2009; Institute of Medicine, 2012). It is clear that ‘prevalence of use’ and ‘benefit the health of the population as a whole’ are requirements at the ‘population level’ that require a product assessment strategy much beyond that described in this series of eight papers. Similarly, a recent review by Hatsukami et al. (2012) on ‘Tobacco and nicotine product testing’ suggests that further studies, in particular on population effects, may be needed to inform a decision on reduced substance exposure. Such evidence should include:

(i) Clinical evaluations using comparator products that are representative of a market sample of different CC. The HPHC yields of the MRTP should ideally, with the exception of nicotine, be below the HPHC yields in CC when expressed on a per mg nicotine basis. Special analytical techniques may be required to identify whether novel compounds are present in the smoke aerosol compared to CC (Knorr et al., 2011).
(ii) Short-term clinical trials that are representative of ‘actual use’, i.e., no limitations in smoking rate, and subjects should be allowed to smoke their preferred brand in the CC group.
(iii) Assessment of consumer acceptability and perceptions of the MRTP.
(iv) Determination of the population exposure of the MRTP as actually used by consumers.
(v) Determination of whether the reduction in exposure from a MRTP vs. CC is ‘substantial’ and supports a potential for reduced risk. A useful approach to this could be the risk and exposure reduction attained with the use of MRTP compared to smoking cessation (or cessation products) in clinical studies (Institute of Medicine, 2012), and
(vi) Estimation of the potential to reduce exposure to HPHC using modeling approaches such as HPHC-to-nicotine correlations (Zenzen et al., 2012) and ‘nicotine bridging’ (Urban et al. 2012).

On the product level, both an MRTP’s aerosol and the conventional cigarette smoke yields to which it is compared was generated in a way that reflects human smoking behavior (taking into account, for example, data from nicotine uptake distributions from clinical or observational studies, in order to better anticipate the exposures that would result from actual product use). Smoking the same MRTP and representative CCs under multiple machine-smoking conditions to determine the HPHC/nicotine ratios over a range of nicotine yields is a novel concept to understand the impact on aerosol composition due to high intra- and inter-smoker variability of nicotine uptake.

We have also studied the performance of the MRTP in a series of clinical studies which compare the use of the product in several different populations. One of the concerns raised by tobacco and public health scientists (Hatsukami et al., 2012) has been that the subpopulation of individuals who may elect to use such products may have specific smoking characteristics which need to be represented in evaluation process. Consequently, populations from three different countries were evaluated using comparator cigarettes with similar ISO tar and nicotine deliveries to the MRTP. A series of clinical studies have been performed which were designed to measure exposure to selected HPHC in a highly controlled environment over a period of several days (Parts 3–6; Tricker et al., 2012a,b,c,d). Such studies are considered appropriate to examine human exposure occurring under natural conditions (Hatsukami et al., 2005). To investigate whether such studies represent real-world patterns of product use, we also investigated biomarkers of exposure and effect in smokers for a one month period under conditions of actual use (Part 7: Martin Leroy et al., 2012).

We have used a panel of biomarkers of exposure to selected HPHC based on the availability of validated analytical methods of determination; however, we realize that some limitations may apply to the selected panel of biomarkers of exposure. The specificity of AAMA and GAMA as biomarkers of exposure to acrylamide in cigarette smoke is limited due to widespread exposure to acrylamide in heat-treated carbohydrate rich foods (Bjellaas et al., 2007). Similarly, the ubiquitous occurrence of acrolein in the environment and endogenous formation during lipid peroxidation (Stevens and Meier, 2008) may limit the usefulness of 3-HPMA to assess changes in tobacco smoke-related exposure to acrolein. Similarly, the specificity of 1-OHP as a surrogate marker for exposure to polycyclic aromatic hydrocarbons (PAH) in cigarette smoke is limited due to multiple environmental sources of pyrene (Strickland et al., 1996). Nevertheless, 1-OHP has proved to be a suitable biomarker of exposure to PAH in studies investigating smoking of either EHCC or conventional cigarettes, and non-smoking, under controlled conditions (Feng et al., 2006). Some doubt also exists as to the specificity of HMPMA as a biomarker of exposure to crotonaldehyde (Hecht et al., 2001). Several known metabolites which have been proposed as biomarkers of exposure to 1,3-butadiene lack sensitivity at low levels of exposure (van Sittert et al., 2000), while many known metabolites of benzene, e.g., trans,trans-muconic acid (t,t-MA), are either non-specific to benzene exposure (Medeiros et al., 1997) or are also present in the diet (Boogaard and van Sittert, 1996; Ruppert et al., 1997). The mainstream smoke constituents responsible for the excretion of mutagenic material in urine are also currently unknown. As a consequence, we have only used the Salmonella YG1024 tester strain which is known to be sensitive to the mutagenic activity of aromatic amino, hydroxylamino, and nitro compounds (Einistö et al., 1990), but is unable to detect the mutagenic activity of other classes of cigarette smoke mutagens excreted in urine.

Our current use of nicotine equivalent excretion in urine, the best available method to estimate total nicotine exposure, has also allowed the determination of effective HPHC-to-nicotine regressions for each of the HPHC determined using biomarkers of expo-
sure. The lowering of toxicants per unit dose of nicotine is considered to be critical by the public health community (Burns, 2006; Burns et al., 2008; World Health Organization, 2008) and has not been adequately addressed in previous studies. The presented series of papers provide clear evidence that this goal can be achieved for many smoke toxicants.

The final paper in this series (Part 8; Urban et al., 2012) offers an approach to bridge from laboratory and clinical studies performed under controlled conditions to estimate exposure at the population level. Although regulatory guidance on the assessment of MRTPs should soon become available in the US (Family Smoking Prevention and Tobacco Control Act, 2009), we present our learnings from reduced exposure testing dating back to before the FSPITCA was enacted. We believe that the elements we present are a step towards a reasonable assessment strategy, but additional insight, in particular for the assessment of population level exposure, needs to be gained from future assessments.

9. Conflict of Interest statement

All authors are Philip Morris International (PMI) R&D employees. The work reported in all eight parts of this supplement was funded by PMI R&D.

References


aspects of regulatory genotoxicity tests for pharmaceuticals S2A, Geneva, Switzerland, ITPMA.


Smoking System. Part 6: 6-day randomized clinical trial of a menthol cigarette in Japan. Regul. Toxicol. Pharmacol. 64 (Suppl. 1), S64–S73.