Regulatory Toxicology and Pharmacology 64 (2012) S85–S97

Contents lists available at SciVerse ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Reduced exposure evaluation of an Electrically Heated Cigarette Smoking System. Part 8: Nicotine bridging – Estimating smoke constituent exposure by their relationships to both nicotine levels in mainstream cigarette smoke and in smokers

H.-Jörg Urban [a], Anthony R. Tricker [a], Donald E. Leyden [b], Natasa Forte [a], Volker Zenzen [c], Astrid Feuersenger [c], Mareike Assink [c], Gerd Kallischnigg [a], Matthias K. Schorp [a,*]

[a] Philip Morris International R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, 2000 Neuchâtel, Switzerland
[b] Hardyville, VA, 23070, USA
[c] Philip Morris International R&D, Philip Morris Research Laboratories GmbH, Fuggerstrasse 3, 51149 Cologne, Germany

ARTICLE INFO

Article history:
Available online 23 August 2012

Keywords:
Biomarkers of exposure
Distribution analysis
Harmful and potentially harmful constituents
HPHC
Nicotine

ABSTRACT

A modeling approach termed 'nicotine bridging' is presented to estimate exposure to mainstream smoke constituents. The method is based on: (1) determination of harmful and potentially harmful constituents (HPHC) and *in vitro* toxicity parameter-to-nicotine regressions obtained using multiple machine-smoking protocols, (2) nicotine uptake distributions determined from 24-h excretion of nicotine metabolites in a clinical study, and (3) modeled HPHC uptake distributions using steps 1 and 2. An example of 'nicotine bridging' is provided, using a subset of the data reported in Part 2 of this supplement (Zenzen et al., 2012) for two conventional lit-end cigarettes (CC) and the Electrically Heated Cigarette Smoking System (EHCSS) series-K6 cigarette. The bridging method provides justified extrapolations of HPHC exposure distributions that cannot be obtained for smoke constituents due to the lack of specific biomarkers of exposure to cigarette smoke constituents in clinical evaluations. Using this modeling approach, exposure reduction is evident when the HPHC exposure distribution curves between the MRTP and the CC users are substantially separated with little or no overlap between the distribution curves.

1. Introduction

Mainstream (MS) cigarette smoke constituent yields are normally quantified and reported using a smoking regimen developed by the Federal Trade Commission (FTC) (Federal Register, 1967), and adopted by the International Organization for Standardization (ISO) (International Organization for Standardization, 2000a,b,c), with minor changes. These two protocols are intended solely to provide comparative information about the level of smoke constituents in different brands of cigarettes by puffing cigarettes according to a convention of analytical standards, but cannot be used to predict smoke constituent uptake by the "average" smoker (Gori and Lynch, 1985; Baker, 2002; Borgerding and Klus, 2005; Federal Trade Commission, 2008a).

MS cigarette smoke consists of an aerosol containing liquid droplets (particulate phase) suspended in the gas–vapor phase, and is generated by overlapping burning, pyrolysis, pyrosynthesis, distillation, sublimation, and condensation processes, with changes in physical and chemical composition of the smoke over time (Baker, 1999; Borgerding and Klus, 2005). Nicotine is mainly present (>99%) in the particulate phase of the MS smoke aerosol (Seeman et al., 2004), and the nicotine dose obtained from a cigarette is subject to substantial inter- and intra-individual differences in smoking behavior (Scherer et al., 2007; Lindner et al., 2011). Smoking a cigarette can be described by distinct physical processes: Puffing, mouth hold, inhalation, and exhalation (Bernstein, 2004). Little buccal absorption of nicotine occurs from acidic smoke of flue-cured tobacco (pH 5.5–6.0), even when held in the mouth (Gori et al., 1986), whereas nicotine from more alkaline air-cured tobacco smoke (pH > 6.5) is efficiently absorbed across the buccal mucosa (Armitage et al., 1978). Regardless of the pH of cigarette smoke, extensive retention (90–100%) of nicotine occurs when the smoke taken into the mouth is inhaled (Robinson and Yu, 2001; Armitage et al., 2004a,b; Feng et al., 2007). Numerous experimental studies have quantified the pulmonary retention of a range of additional HPHC present in both the gas–vapor and particulate phase of MS cigarette smoke which is similar or lower than that of nicotine (Dalhamn et al., 1968; Gori et al., 1986; Armitage et al., 2004a,b; Baker and Dixon, 2006; Feng

* Corresponding author. Fax: +41 58 242 2811.
E-mail address: Matthias.Schorp@pmi.com (M.K. Schorp).

0273-2300/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.yrtph.2012.08.005

et al., 2007; Moldoveanu and St. Charles, 2006; Moldoveanu et al., 2007, 2008a,b,c).

Retained nicotine is extensively metabolized to a number of different metabolites that can be quantified in biological fluids of smokers (Hukkanen et al., 2005; Tricker, 2006). However, because no single smoking machine regimen will adequately reflect human smoking behavior (Institute of Medicine 2001; Baker, 2002; Borgerding and Klus, 2005; World Health Organization Study Group on Tobacco Product Regulation, 2007, 2008), only poor correlations exist between smoke nicotine yields determined using machine-smoking protocols and nicotine-derived biomarker of exposure estimates in smokers (Russell et al., 1980; Rickert and Robinson, 1981; Benowitz and Jacob, 1984; Gori and Lynch, 1985; Diding, 1987; Andersson et al., 1997; Byrd et al., 1998; Jarvis et al., 2001; Ueda et al., 2002; Scherer et al., 2007; Mendes et al., 2009; Lindner et al., 2011). Consequently, the U.S. Federal Trade Commission (FTC) has officially rescinded its guidance for reported smoking machine tar and nicotine yields (Federal Trade Commission, 2008b), while increased interest has occurred within the tobacco control community to develop alternative protocols which better reflect smoker exposure to harmful and potentially harmful constituents (HPHC) (World Health Organization Study Group on Tobacco Product Regulation, 2004, 2007; Hammond et al., 2006, 2007; Marian et al., 2009).

In this study, a 'nicotine bridging' method is described which uses experimentally measured human smoking topography parameters (i.e., puff volume, puff frequency, and puff duration) and 24-h urinary excretion of nicotine metabolites as a measure of nicotine uptake to develop multiple machine smoking protocols to provide smoke nicotine yields that more closely correspond to experimentally determined nicotine uptake estimates in smokers (Urban et al., 2008). This concept is used to evaluate HPHC-to-nicotine and *in vitro* toxicity-to-nicotine relationships for 2 conventional cigarettes (*Marlboro*, *Philip Morris One*) (M6UK, PM1) and the EHCSS-K6 smoked according to ISO and 15 different machine-smoking regimens to reflect 'human puffing behavior' (Schorp et al., 2012; Zenzen et al., 2012). Human smoking behavior was determined using nicotine uptake distributions derived from nicotine metabolite excretion data obtained in two clinical studies (Tricker et al., 2012; Lindner et al., 2011). The two approaches are then combined ('nicotine bridging') to model HPHC uptake proportional to nicotine uptake distributions as a means of assessment of exposure to HPHC for which biomarkers of exposure are not available (Urban and Schorp, 2006).

2. Materials and methods

2.1. Smoking protocols and test and comparator cigarettes

Study cigarettes were analyzed for tar and nicotine according to ISO methods. All study cigarettes were conditioned according to ISO standard 3402 (International Organization for Standardization, 1991). Conventional cigarettes were smoked on smoking machines according to ISO standard 3308 (International Organization for Standardization, 2000a). Tar, nicotine and carbon monoxide (CO) were determined according to IS0 standards 4387, 10315, and 8454, respectively (International Organization for Standardization, 2000b,c, 1995). Mainstream smoke from EHCSS cigarettes was generated on a modified smoking machine with a carousel adapted to use the EHCSS series K lighter. The EHCSS smoke generation conformed to ISO standard 3308, and some slight technical deviations were required. The ISO yields as declared on the cigarette packaging were as follows: *Marlboro* (M6UK; 6 mg tar, 0.5 mg nicotine, and 7.0 mg CO), *Philip Morris One* (PM1; 1 mg tar, 0.1 mg nicotine, and 2.0 mg CO), and EHCSS-K6 (5 mg tar, 0.3 mg nicotine, and 0.6 mg CO). Mainstream smoke was also analyzed for 44 additional HPHC according to ISO conditions plus 15 experimental smoking regimens reflecting 'human puffing behavior' (Zenzen et al., 2012). The data used represent a subset of the data set reported by Zenzen et al. (2012) to illustrate the principle of the 'nicotine bridging' method.

2.2. HPHC yields and in vitro toxicological parameters

Five "toxicological parameters" were determined for mainstream smoke total particulate matter (TPM) using the *Salmonella typhimurium* reverse mutation assay with tester strains TA98, TA100, and TA1537 with S9 metabolic activation (Organization for Economic Co-operation and Development, 1997), and the neutral red uptake (NRU) assay for both mainstream smoke gas-vapor phase (GVP) and TPM (Borenfreund and Puerner, 1985).

2.3. Biomarker measurements in urine

Urinary excretion of biomarkers of exposure to nicotine and selected cigarette smoke HPHC (1,3-butadiene, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone [NNK], acrolein, benzene, CO, pyrene, and *o*-toluidine) were determined in a clinical study comparing exposure of smokers ($N = 32$ smokers per group) of the M6UK, PM1, and EHCSS-K6 cigarettes (Tricker et al., 2012). Biomarkers of exposure were determined in 24 h urine samples collected on study days 3–8 after randomization to the test cigarettes. Nicotine uptake was expressed as excretion of nicotine equivalents in 24 h urine (Neq; the calculated molar sum of the concentrations of nicotine, cotinine, *trans*-3′-hydroxycotinine, and their respective glucuronide conjugates). Nicotine uptake, expressed as 24 h urine excretion of Neq, was also determined in a European population of smokers from the UK, Germany, and Switzerland (Lindner et al., 2011). Smokers were randomized according to different cigarette ISO tar categories (TC): TC1 (1–4 mg ISO tar; $N = 409$), TC2 (5–7 mg ISO tar, $N = 399$), and TC3 (8–12 mg ISO tar, $N = 387$).

2.4. 'Nicotine bridging' method

The nicotine bridging method uses a three-step approach as shown in Fig. 1. This approach is based on: (1) MS smoke HPHC or *in vitro* toxicity parameter-to-nicotine regressions obtained using multiple machine smoking protocols, (2) nicotine uptake distributions from clinical studies, and (3) modeled HPHC uptake distributions (Urban and Schorp, 2006). For a 5 days average estimate of nicotine uptake, determined as Neq excretion per day for each smoker, the corresponding smoke constituent uptake was estimated based on the corresponding regression equation (i.e., using the slope and the intercept of the HPHC-to-nicotine yield). In this way, a modeled HPHC uptake value for each smoker was obtained which can be displayed as a frequency distribution for all smokers. This method is based on the underlying assumptions that (i) proportionality exists between the yield of different HPHC to nicotine (Zenzen et al., 2012), and (ii) that uptake of each HPHC is proportional to the nicotine uptake distribution. The latter is a rather conservative assumption as almost complete uptake of nicotine occurs when cigarette smoke reaches the small airways of the lung, while the retention of other HPHC may be lower (Robinson and Yu, 2001; Armitage et al., 2004a,b; Feng et al., 2007).

For each HPHC/*in vitro* toxicity parameter the intercept (a) and the slope (b) of the corresponding regression equation was determined using standard linear regression analysis. The coefficient of determination r^2 was used to assess the quality of the linear relationship. Examples of graphical representations of HPHC-to-nicotine relationships and further interpretation are reported by Zenzen et al. (2012).