Research Article

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Comparison of Exposure to Selected Cigarette Smoke Constituents in Adult Smokers and Nonsmokers in a European, Multicenter, Observational Study

Dirk Lindner[1], Stephen Smith[3], Claire Martin Leroy[2], and Anthony R. Tricker[1]

Abstract

Background: This multicenter, observational study was conducted in three European countries (Germany, Switzerland, and the United Kingdom) to determine the exposure of adult cigarette smokers and nonsmokers to selected cigarette smoke constituents: 1,3-butadiene, 2-naphthylamine, 4-aminobiphenyl, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), acrolein, benzene, carbon monoxide, nicotine, pyrene, and *o*-toluidine.

Methods: Smokers were grouped by tar category (TC) according to the tar yield of their regular cigarette brand: TC1: ≤4 mg tar, TC2: 5–7 mg tar, and TC3: ≥8 mg tar [to the legal tar yield ceiling in the respective countries (10 or 12 mg tar)]. Levels of biomarkers of exposure to the aforementioned cigarette smoke constituents were compared between smokers and nonsmokers, and within smokers across tar categories.

Results: The full population consisted of 1,631 subjects (1,223 smokers and 408 nonsmokers). Biomarkers of exposure were analyzed for 1,558 subjects (valid case population) as follows: 1,159 smokers (TC1: $n = 402$, TC2: $n = 379$, TC3: $n = 378$), and 399 nonsmokers. Exposure levels were higher in smokers than nonsmokers and increased with increasing tar yield and cigarette consumption. An association of tar category and exposure level was observed for all smoke constituents, except pyrene, 4-aminobiphenyl, and *o*-toluidine, whereas only NNK exposure was different in all three tar categories.

Conclusions: Smoking status and, among smokers, daily cigarette consumption and tar yield were observed to affect biomarker of exposure levels.

Impact: This research provides a comprehensive evaluation of smoke constituent exposure of adult cigarette smokers and nonsmokers in three European countries. *Cancer Epidemiol Biomarkers Prev; 20(7); 1524–36. ©2011 AACR.*

Introduction

Epidemiologic evidence suggests that a relationship exists between both the amount smoked and the duration of smoking, and the risk of cigarette smoking–related diseases (1). However, the relationship between a smoker's exposure to potentially harmful smoke constituents and the risk of smoking-related diseases is unclear.

Determinations of mainstream smoke constituents using the Federal Trade Commission–standardized machine-smoking protocol (2), or the similar International Organization for Standardization (ISO) method (3), can provide comparative information about the level of smoke constituents generated by different brands of cigarettes according to machine-smoking conditions (4). The major limitation of these machine-driven yields is that they do not represent actual human-smoking behaviors (5) and, therefore, they cannot account for the complex parameters that will affect an individual's exposure (6–10).

With a well-established body of evidence linking cigarette smoking to lung cancer, myocardial infarction, stroke, and chronic obstructive pulmonary disease (1, 11, 12), a better measure of the uptake of smokers to the constituents in cigarette smoke is needed.

The World Health Organization (WHO) has published its second report on Tobacco Regulation toward the mandated lowering of the levels of toxicants in tobacco (13). Insight into biomarkers of exposure to tobacco smoke constituents in human subjects can provide greater knowledge about exposure of individuals to harmful constituents and the assessment of products aimed at reducing this exposure (14).

Authors' Affiliations: [1]Philip Morris International R&D, Philip Morris Products S.A., Neuchâtel; [2]Clinopsis S.A., Concise, Switzerland; and [3]Celerion, Belfast, Northern Ireland

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

Corresponding Author: Dirk Lindner, Clinopsis S.A., Jardins 6, 1426 Concise, Switzerland. Phone: 41 (24) 5340191; E-mail: dirk.lindner@clinopsis.com

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This study was intended to extend the knowledge about exposure of adult smokers and nonsmokers in a European population to harmful cigarette smoke components, carcinogens in particular, and to provide additional information about exposure of smokers with regard to cigarette tar yields.

Materials and Methods

Study design

This was an observational, parallel-group, multicenter study conducted in 3 countries, at 5 sites in the United Kingdom, 5 sites in Germany, and 2 sites in Switzerland, to assess the exposure of adult smokers and nonsmokers to selected cigarette smoke constituents in a real-life setting.

Smokers were assigned to 3 groups according to the ISO tar yield of their current regular commercial cigarette brands. Smoking groups were defined as tar category 1 (TC1): tar yields 4 mg or less; TC2: tar yields of 5 to 7 mg; or TC3: 8 to 12 mg (10 mg tar in Germany and the United Kingdom, and 12 mg tar in Switzerland, corresponding to the upper tar yield ceilings in the respective countries[1]).

This study was conducted in compliance with the ethical principles that have their origin in the Declaration of Helsinki and in accordance with Good Clinical Practice (15, 16). Before study commencement, the protocol, informed consent forms, and all advertisements used for subject recruitment were reviewed and approved by research ethics committees in the participating countries.

Study participants

Eligible subjects were healthy males or females, 21 years of age or older, either nonsmokers who had not used any tobacco or nicotine-containing product for at least 1 year before screening (visit 1) or regular smokers of at least 1 cigarette per day (cig/d). Smokers should have exclusively smoked nonmentholated commercial cigarettes, should not have used any other nicotine-containing product, and should not have changed their regular cigarette brand within 3 months prior to study enrollment. Subjects could not participate in this study if they had donated or received blood products or participated in another clinical trial within 3 months before screening. Participants with a positive drug-screening result or who showed signs of alcohol abuse were excluded from the study. Female participants were excluded from the study if they were pregnant or nursing, had not used reliable birth control in the cycle before the study, or did not agree to continue using reliable birth control during the study.

Subjects were recruited from each investigational site's geographic area through newspaper (in all countries) and radio (United Kingdom only) advertisements. All subjects were screened at the investigational site. In the United Kingdom, a prescreening was conducted through a central call center. Screening procedures at investigational sites were conducted only after the subjects had signed an informed consent form. Subjects were compensated for their participation in the study in accordance with local compensation practices. Subjects were advised that they were allowed to withdraw from the study at any time and would receive a prorated stipend. Withdrawn subjects could not reenter the study and were replaced. Smoking subjects were advised on the risks of smoking and counseled as appropriate. All smokers were free to stop smoking at any time during the study without being excluded from the study.

Procedures

Subjects were asked to report to the study site 3 times: for screening (visit 1) and for study assessments [visit 2 (5–14 days following visit 1) and visit 3 (3–5 days following visit 2)]. One of the 2 assessment visits was scheduled on the weekend (Saturday or Sunday), the other one on a weekday (Tuesday through Friday).

At visit 1, subjects gave informed consent and screening assessments were conducted. Eligible subjects completed a self-administered questionnaire asking for information on smoking history, including the Fagerström Test for Nicotine Dependence (FTND; ref. 17).

Smokers were assigned to a tar category on the basis of the tar yield of their current brand of cigarette at visit 1. Subjects smoked their own purchased cigarettes and were allowed to smoke *ad libitum* but were asked to avoid altering their smoking patterns or brand of cigarettes during the course of the study. Smokers had to document the time and brand of all cigarettes smoked during the study (from 2 days prior to visit 2 until visit 3), using an electronic diary (PHT LogPad; PHT Corporation). In addition, the brand name and tar and nicotine yields of the smokers' current cigarette brand were recorded at each study visit.

Nonsmokers were asked to record the daily duration of their exposure to environmental tobacco smoke (ETS) using the same electronic diary. Smokers did not have to record their exposures to ETS.

Blood samples for assessment of biomarkers of exposure were taken at visits 2 and 3 (scheduled for 6:00 PM ± 2 hours). Study participants were asked to collect urine for consecutive 24 hours, beginning between 7:00 AM and 12 PM on the days prior to visits 2 and 3, for measurements of urinary biomarkers of exposure. Urine samples had to be kept in cool bags from the start of collection

[1]According to the Directive 2001/37/EC of the European Parliament and of the Council of 5 June 2001 on the approximation of laws, regulations and administrative provisions of the Member States concerning the manufacture, presentation, and sale of tobacco products, the maximum ISO tar yield limit was 10 mg in Germany and the United Kingdom.According to the Verordnung über Tabakerzeugnisse und Raucherwaren mit Tabakersatzstoffen, Der Schweizerisches Bundesrat, October 27, 2004, the maximum ISO tar yield limit in Switzerland was reduced to 10 mg, effective as of November 1, 2004. According to Article 21 of this ordinance, the selling of cigarettes according to the previous ordinance of March 1, 1995, that is, of cigarettes with an ISO tar yield of up to 15 mg, was allowed until April 30, 2006. For this study, conducted from April 2005 to May 2006,12 mg ISO tar was selected as the maximum tar yield for smokers in Switzerland, thus excluding about 1.5% of the smoking Swiss population.

until return to the site. During urine collection, smokers were required to collect at least 5 cigarette butts (or all if <5 were smoked) for cigarette butt analysis.

Bioanalytic methodology

Urinary biomarkers of exposure were nicotine and 5 metabolites: nicotine equivalents (Neq) for nicotine (18), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronide conjugates (total NNAL) for 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK; ref. 19), monohydroxybutenyl mercapturic acid (MHBMA) for 1,3-butadiene (20), 3-hydroxypropylmercapturic acid (3-HPMA) for acrolein (21), *S*-phenylmercapturic acid (*S*-PMA) for benzene (22), and 1-hydroxypyrene and its glucuronide and sulfate conjugates (total 1-OHP) for pyrene (23). Urinary concentrations of 2-naphthylamine (2-NA), 4-aminobiphenyl (4-ABP), and *o*-toluidine (*o*-TOL) were directly measured (24). For all urinary biomarkers of exposure, the concentrations in 24-hour urine samples were determined by liquid chromatography/tandem mass spectrometry (LC/MS-MS). Carboxyhemoglobin (COHb), as the biomarker for carbon monoxide, was measured in blood by spectrophotometry, and plasma cotinine (PCOT), an additional biomarker for nicotine, was determined by LC/MS-MS. The LC/MS-MS methods used to determine the urinary biomarkers of exposure and PCOT were validated according to Food and Drug Administration criteria (25).

Cigarette butt analysis

The nicotine content in a 10-mm portion of the mouth end of the cigarette filter (26) and the cigarette butt length were determined from cigarette butts collected during the 24-hour urine collection period.

Safety assessments

At visit 1, participants' medical history and concomitant medications were recorded; physical examination, vital signs assessment, and clinical laboratory assessments were conducted. Vital signs assessment was repeated at visit 3. Concurrent illnesses were recorded at all visits. Female participants of child-bearing potential were required to undergo a pregnancy test at visits 1 and 2.

Statistical analysis

It was assumed that 360 subjects in each tar category group (120 per group in each of the 3 countries) would be sufficient to detect differences in the Neq [amount excreted in 24 hours (Ae24h)] levels between the lowest (TC1) and highest (TC3) tar categories. In general, without adjustment for multiplicity, this sample size is sufficient to detect a difference of 0.21σ (σ = common SD) between 2 groups at the 5% significance level with a power of 80% (2-sided Student's *t* test). An interim analysis, based on 160 subjects of TC1 and TC3 (80 each), was planned and conducted to estimate the actual detectable difference for Neq. According to the results of that interim analysis (observed σ was 7.33 mg/24 h), with the planned sample size of 360 subjects per tar category, a difference in Neq Ae24h levels between TC3 and TC1 of -1.36 mg/24 h $= 0.19\sigma$ was expected to be detected at the 5% significance level with a power of 80%.

The statistical analysis of the study data was conducted according to a statistical analysis plan, developed prior to database lock and prior to disclosure of the subjects' allocation to the tar categories.

Two analysis populations were defined: the full population, comprising all subjects enrolled and who attended at least one of the visits (visit 2 or 3), and the valid case population, consisting of all subjects with at least 1 valid biomarker measurement at visit 2 or 3, and without major protocol deviations. In the valid case analysis, blood samples collected outside the time window from 3:00 PM to 9:00 PM (and the corresponding COHb and PCOT values) and values of urinary biomarkers from potentially invalid 24-hour urine samples (i.e., with volume <500 mL, or not, collected over 20–28 hours, or with urine creatinine levels <500 or 700 mg/24 h in females and males, respectively) were excluded from the analysis. Potentially misclassified subjects, both smokers and nonsmokers, were identified by biologically implausible (very low or very high) levels of biomarkers of exposure for nicotine (27) and total NNAL (19) and were also excluded from the valid case analysis. Demographic and safety data were analyzed on the basis of the full population; biomarkers of exposure data were analyzed on the basis of the valid case population.

The allocation of smokers to tar categories was initially done at visit 1. This allocation was verified and, if necessary, adjusted to the cigarette brand smoked most frequently according to the records in the electronic diary. Nonsmokers were stratified according to their average daily exposure to ETS ($\leq$30 min/d vs. >30 min/d) as recorded in the electronic diary.

For the biomarkers of exposure measured in blood or plasma (COHb, PCOT), mean concentration values were used for analysis. In addition, in smokers, these concentrations were adjusted by the number of cigarettes smoked during the visit day between 6:00 AM and blood sampling (6:00 PM $\pm$ 2 hours).

For the urinary biomarkers of exposure (see Table 1), the Ae24h and the Ae24h adjusted by the amount creatinine excreted in 24 hours were derived from the measured concentrations. In addition, both derived values were adjusted for the total number of cigarettes smoked during the urine collection period to estimate the biomarker levels per cigarette.

For the purpose of the analysis, biomarkers of exposure concentrations below the lower limit of quantification (LLOQ, Table 1) were replaced as follows: for smokers and nonsmokers exposed to ETS during the 24-hour urine collection period for more than 30 minutes by 0.5 $\times$ LLOQ; for nonsmokers exposed to ETS for less than 30 minutes during that period by 0.

Table 1. Biomarkers of exposure: smoke constituents and analytic methods

Biomarkers of exposure	Matrix	Smoke constituent	Analytic method	LLOQ
COHb	Blood	Carbon monoxide	Spectrophotometry	1%
PCOT	Plasma	Nicotine	LC/MS-MS	10 ng/mL
Neq	Urine[a]	Nicotine	LC/MS-MS	10 ng/mL (for each metabolite)
2-NA	Urine[a]	2-NA	LC/MS-MS	5 pg/mL
4-ABP	Urine[a]	4-ABP	LC/MS-MS	5 pg/mL
3-HPMA	Urine[a]	Acrolein	LC/MS-MS	35 ng/mL
MHBMA	Urine[a]	1,3-Butadiene	LC/MS-MS	0.105 ng/mL
S-PMA	Urine[a]	Benzene	LC/MS-MS	20 pg/mL
Total 1-OHP	Urine[a]	Pyrene	LC/MS-MS	10 pg/mL
Total NNAL	Urine[a]	NNK	LC/MS-MS	5 pg/mL
o-TOL	Urine[a]	*o*-TOL	LC/MS-MS	25 pg/mL

[a]Twenty-four hour urine sample.

Data analysis was conducted on the basis of the averages of visits 2 and 3. If subjects reported only to 1 of the 2 visits, the levels reported at the particular visit were included in the data analysis. Descriptive statistics [number of nonmissing observations (n), mean, SD, and minimum and maximum values, quartiles] were calculated for all biomarkers of exposure analysis variables described previously. Data were stratified by study groups, country, gender, and daily cigarette consumption.

Inferential statistical analyses were conducted for the unadjusted biomarker variables only, that is, COHb and PCOT concentration and Ae24h values for all urinary biomarkers of exposure. Results from the inferential analyses were interpreted at the significance level of $\alpha = 0.05$ (2-sided). Differences in biomarker of exposure levels between smokers and nonsmokers were tested using Wilcoxon signed rank-sum tests. Differences in exposure to smoke constituents between the 3 tar categories were examined using ANOVA, with adjustments by the factors of country, age (21–34, 35–49, and $\geq$50 years), gender, and daily cigarette consumption (<10 cig/d, 10–19 cig/d, and $\geq$20 cig/d). Adjusted least-squares means with 95% CIs for each tar category, accounting for any imbalance between tar categories with regard to those factors, and differences of least-square means with simultaneous 95% CIs (Tukey's method) were calculated. Further adjustments for multiplicity were not considered necessary, as this study was not designed to be confirmatory of any hypothesis, and all analyses were considered to be exploratory. In a post-hoc analysis, the body mass index (BMI), classified as <25 kg/m^2 (underweight and normal weight) and $\geq$25 kg/m^2 (overweight and obesity), was included in the ANOVA models to examine the effect of the BMI on the biomarker levels.

All analyses were conducted using SAS Version 8.2 (SAS Institute Inc.).

Results

Study population

An overview of subject disposition over the study is presented in Supplementary Figure S1. In total, 1,667 subjects were enrolled into the study from April 2005 to May 2006. Thirty-six subjects dropped out at the screening visit without further follow-up. Thus, 1,631 subjects (1,223 smokers and 408 nonsmokers) completed the screening visit and continued after visit 1: 572 subjects in the United Kingdom (429 smokers and 143 nonsmokers); 700 subjects in Germany (527 smokers and 173 nonsmokers); and 359 subjects in Switzerland (267 smokers and 92 nonsmokers).

A total of 1,584 subjects (1,179 smokers and 405 nonsmokers) completed the study, and 47 subjects (44 smokers and 3 nonsmokers) withdrew prior to visit 3. The most common reasons for withdrawal were withdrawal of consent, lost to follow-up, and noncompliance to study procedures.

For 43 subjects, major protocol deviations were reported: 37 subjects smoked mentholated cigarettes, 6 subjects had a violation of selection criteria for health status, and 1 subject was employed by the study site (1 subject had 2 major protocol deviations).

The full population comprised all 1,631 subjects enrolled: 1,223 smokers (TC1, 418; TC2, 407; and TC3, 398) and 408 nonsmokers. A total of 73 subjects were excluded from the valid case population: 43 subjects with major protocol deviations, 27 subjects without valid biomarker of exposure measurements, and 3 subjects (all nonsmokers) who were judged as potentially misclassified on the basis of their nicotine biomarker levels. Thus, the valid case population consisted of 1,558 subjects: 1,159 smokers (TC1, 402; TC2, 379; TC3, 378) and 399 nonsmokers.

Demographic data

Of the 1,631 subjects in the full population, 957 subjects (58.7%) were female and 674 subjects (41.3%) were male.

Table 2. Demographic data, smoking history, and daily cigarette consumption by smoking status (valid case population)

Variable	Smokers				Nonsmokers	All
	TC1	TC2	TC3	All smokers		
N	402	379	378	1,159	399	1,558
Age, y						
Mean (SD)	36.0 (11.7)	34.5 (11.2)	37.0 (11.8)	35.9 (11.6)	44.0 (15.8)	37.9 (13.3)
Median	34	32	36	34	41	35
Range	21–78	21–72	21–72	21–78	21–85	21–85
Gender, *n* (%)						
Male	136 (33.8)	136 (35.9)	189 (50.0)	461 (39.8)	192 (48.1)	653 (41.9)
Female	266 (66.2)	243 (64.1)	189 (50.0)	698 (60.2)	207 (51.9)	905 (58.1)
BMI, kg/m^2						
Mean (SD)	24.5 (4.1)	24.6 (4.4)	25.3 (4.7)	24.8 (4.4)	25.6 (4.6)	25.0 (4.5)
Median	24.0	23.5	24.6	24.0	24.9	24.3
Range	16.9–40.8	16.8–46.8	16.8–48.4	16.8–48.4	16.4–51.3	16.4–51.3
Duration of smoking, *n* (%), y						
<1	4 (1.0)	1 (0.3)	0	5 (0.4)	–	–
1–10	159 (40.2)	180 (47.6)	17 (28.5)	446 (38.8)	–	–
11–20	115 (29.0)	108 (28.6)	120 (32.0)	343 (29.9)	–	–
>20	118 (29.8)	89 (23.5)	148 (39.5)	355 (30.9)	–	–
FTND score						
Mean (SD)	3.0 (2.4)	3.0 (2.6)	4.1 (2.4)	3.4 (2.5)	–	–
Median	3	3	4	3	–	–
Range	0–10	0–10	0–10	0–10	–	–
Daily cigarette consumption,[a] cig/d						
Mean (SD)	10.4 (5.8)	11.0 (6.4)	13.7 (6.2)	11.7 (6.3)	–	–
Median	10	10	13	11	–	–
Range	1–37	1–39	2–35	1–39	–	–
<10 *n* (%)	192 (47.8)	179 (47.2)	96 (25.4)	467 (40.3)	–	–
10–19, *n* (%)	180 (44.8)	157 (41.4)	220 (58.2)	557 (48.1)	–	–
≥20, *n* (%)	30 (7.5)	43 (11.3)	62 (16.4)	135 (11.6)	–	–
Nonsmoker status						
Never smoker, *n* (%)	–	–	–	–	252 (64.0)	–
Ex-smoker, *n* (%)	–	–	–	–	142 (36.0)	–

NOTE: *N*, number of subjects in group; *n*, number of subjects in class; TC1, ≤4 mg tar; TC2, 5–7 mg tar; TC3, ≥8 mg tar.
[a]Daily cigarette consumption during the study (median of self-reported daily consumption from 2 days prior to visit 2 until visit 3).

Among the smokers enrolled, the proportion of females ($n = 749$, 61.2%) was higher than males ($n = 454$, 38.8%). In the nonsmoker group, the proportion of males ($n = 200$, 51.0%) and females ($n = 208$, 49.0%) was comparable. The mean age of all subjects was 38.1 ± 13.3 years and similar by gender. The mean age of nonsmokers (44.3 ± 15.9 years) was higher than that of smokers (36.0 ± 11.6 years), with the mean age of smokers in the 3 tar groups being comparable. The mean age of smokers with a daily cigarette consumption of 20 to 30 cigarettes (39.1 years; $n = 130$) or more than 30 cigarettes (40.0 years; $n = 13$) was higher than that of subjects who smoked either 10 to 19 cigarettes (34.6 years; $n = 591$) or less than 10 cigarettes (37.6 years; $n = 489$). The mean age was higher in subjects from the United Kingdom (43.9 ± 15.4 years) than from those in Germany (36.3 ± 11.6 years) and Switzerland (32.3 ± 8.6 years). Demographic data of the full population and the valid case population (see Table 2) were similar.

Smoking history

The reported duration of smoking prior to enrollment was 1 to 10 years for 460 smokers (38.0%), 11 to 20 years for 366 smokers (30.2%), and more than 20 years for 377 smokers (31.2%). Seven smokers (0.6%) reported smoking less than 1 year. The mean FTND score in smokers was 3.4 ± 2.5 and was higher in the TC3 group (4.1 ± 2.4) than in the TC2 and TC1 groups (3.0 ± 2.6 and 3.0 ± 2.5, respectively). This score was higher in the United Kingdom (4.0 ± 2.6) than in Germany (3.0 ± 2.4) and Switzerland (3.2 ± 2.4). There were no marked differences found

between male and female smokers. Smoking history data for the valid case population can be found in Table 2.

Of the 408 nonsmokers, 258 (64.0%) reported that they were never-smokers and 145 (36.0%) reported being ex-smokers (no information available for 5 subjects), with similar distributions by age and in the 3 countries. For ex-smokers, the mean time from smoking cessation to enrollment was 14.7 ($\pm$ 12.3) years, with a range of 1 to 56 years.

Cigarette consumption

The average daily cigarette consumption of smokers from the valid case population during the study was 11.7 $\pm$ 6.3 cig/d: 467 smokers usually smoked less than 10 cig/d, 557 smokers smoked 10–19 cig/d, and 135 smokers smoked 20 or more cig/d (of which only 10 smoked >30 cig/d on average). In general, smokers of cigarettes with higher tar yields tended to have higher daily cigarette consumption (Table 2). About 10% of the smokers had a usual daily cigarette consumption of less than 5 cig/d, consistently across all tar categories. Slightly higher cigarette consumption on the weekends (13.2 $\pm$ 6.9 cig/d) than on weekdays (12.6 $\pm$ 6.8 cig/d) was observed. The average number of cigarettes smoked prior to the COHb assessment was 6.7 $\pm$ 3.9 cig and, in contrast to the trend for the daily consumption, was slightly higher on weekdays (7.2 $\pm$ 4.2 cig) than on weekends (6.6 $\pm$ 4.1 cig). These trends were observed similarly in all 3 TC groups.

Exposure of nonsmokers to ETS

Of the 399 nonsmokers, 205 (51.5%) reported being exposed to ETS during the study on average more than 30 min/d and 193 subjects (48.5%) reported being exposed to ETS for less than 30 min/d. The mean duration of exposure to ETS of nonsmokers prior to the study visits was higher on weekends (1.05 $\pm$ 1.86 h/d) than on weekdays (0.68 $\pm$ 1.58 h/d), with an overall average of 0.86 $\pm$ 1.50 h/d.

Biomarkers of exposure

Subjects of the valid case population who did not have reliable data for either blood or urinary biomarkers of exposure were excluded from that particular analysis. Thus, urinary biomarkers of exposure were analyzed for 1,556 subjects (1,158 smokers and 398 nonsmokers), and blood/plasma biomarkers of exposure were analyzed for 1,549 subjects (1,150 smokers and 399 nonsmokers).

For all biomarkers of exposure assessed in this study, high variability was observed within each of the 4 study groups; the absolute variability was much smaller in the nonsmoker group, but the relative variability was as high as in the smoker groups. As expected, levels of all biomarkers of exposure were distinctly lower in nonsmokers than in smokers ($P < 0.001$; Table 3), with the largest differences observed for Neq, PCOT, and total NNAL (Table 4). Figures 1–3 show urinary 24-hour excretion (Ae24h) for Neq, total NNAL, and evening COHb saturation in blood for smokers (by tar category and daily cigarette consumption) and nonsmokers. Table 3 provides the results of the inferential statistical analyses (a) comparing BoExp levels between nonsmokers and smokers and (b) testing for association of tar yield and exposure of smokers adjusting for the factors country, age, gender, and daily cigarette consumption.

Biomarkers of exposure and tar yield

In general, levels of biomarkers of exposure increased with tar yield (Tables 3 and 4). For all biomarkers of exposure, the highest levels were observed in the TC3 group and, for all biomarkers except 4-ABP and *o*-TOL, the lowest were found in the TC1 group (lowest 4-ABP and *o*-TOL levels were measured in TC2).

Results of the inferential statistical analyses adjusting for the factors country, age, gender, and daily cigarette consumption in smokers suggest an association of tar yield and exposure for COHb, PCOT, Neq, 2-NA, 3-HPMA, MHBMA, *S*-PMA, and total NNAL ($P \leq 0.05$; Table 3); however, the partial contribution of the factor tar category to the total variation was not more than 2% (partial $R^2 \leq 0.02$) for all biomarkers. For all these biomarkers, statistically significant ($P \leq 0.05$) differences between the lowest and highest tar categories, TC1 and TC3, were observed. Only for total NNAL, statistical tests suggest statistically significant differences between all 3 tar categories. In addition, for COHb, Neq, 2-NA, and 3-HPMA, differences between TC1 and TC2 and for PCOT a difference between TC2 and TC3 were observed. The findings were similar after adjustment for urinary creatinine.

Biomarkers of exposure and cigarette consumption

Mean levels of all biomarkers of exposure appeared to be strongly associated with cigarette consumption, increasing with higher consumption ($P \leq 0.05$; Tables 3 and 5). In general, the highest levels for all biomarkers of exposure were observed in smokers with a daily consumption of 20 or more cig/d; the lowest levels in smokers with a daily consumption of less than 10 cig/d (Table 5). Although considered to be a significant factor in all models, the contribution of that factor to the total variation was small for 2-NA, 4-ABP, total 1-OHP, and *o*-TOL (partial $R^2 < 0.03$) whereas for 3-HPMA, MHBMA, *S*-PMA, and total NNAL, the partial R^2 ranged from about 0.13 to 0.17 and for COHb, PCOT, and Neq, the partial R^2 ranged from about 0.24 to 0.30. Analysis of biomarkers of exposure levels adjusted for the number of cigarettes smoked (during urine collection interval for urinary biomarkers and between 6:00 AM and blood sampling for COHb and PCOT, respectively) suggested that for all biomarkers of exposure, except 3-HPMA, the means of the per-cigarette levels decreased with increasing daily cigarette consumption (Supplementary Table S1).

Biomarkers of exposure and gender, age, BMI, and country effects

For all biomarkers of exposure, male smokers appeared to have higher mean levels than female smokers (Supplementary Table S2). For all biomarkers except COHb,

Table 3. Biomarkers of exposure—results from inferential analysis (valid case population)

Biomarker	Smokers vs. nonsmokers[a]	Results from within-smoker analysis (ANOVA for effect of TC, adjusted for consumption, age, gender, and country)								
		Least-square means,[b] (95% CI) by TC			*P* for fixed effects included in the ANOVA					R^2
		TC1	TC2	TC3	TC	cig/d	Age	Gender	Country	
Concentration at 6:00 PM ± 2 h										
COHb, %	<0.0001	3.99 (3.80–4.18)	4.41 (4.21–4.60)	4.53 (4.34–4.71)	<0.0001[c,e]	<0.0001	<0.0001	0.6553	<0.0001	0.4696
PCOT, ng/mL	<0.0001	226.8 (214.7–239.0)	243.5 (231.1–255.8)	274.1 (262.3–285.9)	<0.0001[d,e]	<0.0001	<0.0001	0.0163	0.0023	0.3986
Ae24h										
Neq, mg/24 h	<0.0001	11.14 (10.48–11.80)	12.60 (11.93–13.28)	13.59 (12.95–14.24)	<0.0001[c,e]	<0.0001	<0.0001	<0.0001	0.2656	0.3976
2-NA, ng/24 h	<0.0001	13.93 (10.49–17.37)	20.62 (17.12–24.11)	22.44 (19.10–25.79)	0.0002[c,e]	<0.0001	0.0019	0.0282	0.0049	0.0867
4-ABP, ng/24 h	<0.0001	27.51 (18.85–36.16)	26.58 (17.78–35.37)	32.15 (23.73–40.57)	0.5677	0.0012	0.0231	0.1960	0.1685	0.0303
3-HPMA, mg/24 h	<0.0001	1.94 (1.82–2.06)	2.18 (2.06–2.30)	2.29 (2.18–2.41)	<0.0001[c,e]	<0.0001	<0.0001	<0.0001	<0.0001	0.3436
MHBMA, μg/24 h	<0.0001	3.54 (3.23–3.85)	3.95 (3.64–4.27)	4.26 (3.96–4.56)	0.0012[e]	<0.0001	0.0083	<0.0001	0.1487	0.2240
S-PMA, μg/24 h	<0.0001	4.48 (4.13–4.82)	4.83 (4.48–5.18)	5.22 (4.89–5.56)	0.0030[e]	<0.0001	0.0018	0.0312	0.0559	0.2322
Total 1-OHP, ng/24 h	<0.0001	307.7 (267.9–347.6)	326.4 (285.9–366.9)	347.4 (308.6–386.1)	0.2955	<0.0001	0.0824	0.0038	0.6830	0.0581
Total NNAL, ng/24 h	<0.0001	204.7 (188.9–220.5)	227.8 (211.7–243.8)	260.6 (245.2–275.9)	<0.0001[c–e]	<0.0001	<0.0001	0.0020	0.0015	0.2936
o-TOL, ng/24 h	<0.0001	183.4 (126.4–240.3)	175.4 (117.6–233.2)	240.4 (184.8–296.1)	0.1587	0.0229	0.8498	0.7999	0.1384	0.0169

NOTE: R^2 of ANOVA model with factors tar category, daily cigarette consumption, age, gender, and country.

[a]Smokers versus nonsmokers, *P* value from Wilcoxon signed rank-sum test.

[b]Least-square means for the factor tar category, adjusted for the average value of factors daily cigarette consumption, age, gender, and country.

[c–e]Statistically significant differences ($P < 0.05$) in least-square means between (c) TC1 and TC2, (d) TC2 and TC3, and (e) TC1 and TC3 (Tukey's method).

Table 4. Biomarkers of exposure by tar yield (unadjusted values, valid case population)

Biomarker	Smokers								Nonsmokers	
	TC1 ($N = 402$)		TC2 ($N = 379$)		TC3 ($N = 378$)		All smokers ($N = 1,159$)			
	n	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)
Concentration at 6:00 PM ± 2 h										
COHb, %	396	3.12 (2.11)	376	3.53 (2.31)	376	4.28 (2.06)	1,148	3.63 (2.21)	398	0.23 (0.40)
PCOT, ng/mL	397	177.1 (131.4)	377	193.7 (130.6)	376	261.7 (125.4)	1,150	210.2 (134.2)	398	2.23 (9.11)
Ae24h										
Neq, mg/24 h	401	8.56 (6.87)	379	10.13 (7.04)	375	13.33 (7.23)	1,155	10.62 (7.31)	396	0.05 (0.34)
2-NA, ng/24 h	401	11.50 (18.69)	379	18.14 (42.50)	376	23.37 (25.87)	1,156	17.54 (30.88)	395	1.79 (3.59)
4-ABP, ng/24 h	401	21.72 (87.81)	379	20.45 (17.14)	376	32.05 (94.47)	1,156	24.66 (75.44)	397	2.77 (11.79)
3-HPMA, mg/24 h	401	1.48 (1.15)	379	1.75 (1.24)	376	2.22 (1.30)	1,156	1.81 (1.26)	398	0.63 (0.80)
MHBMA, μg/24 h	401	2.64 (2.75)	379	3.11 (3.07)	376	4.11 (3.07)	1,156	3.27 (3.02)	398	0.30 (0.42)
S-PMA, μg/24 h	401	3.49 (3.29)	379	3.90 (3.29)	376	5.03 (3.34)	1,156	4.13 (3.36)	398	0.39 (0.27)
Total 1-OHP, ng/24 h	401	258.1 (515.1)	379	276.2 (207.0)	376	339.8 (228.4)	1,156	290.6 (352.2)	398	122.5 (100.5)
Total NNAL, ng/24 h	400	150.8 (139.5)	379	173.6 (137.0)	376	246.8 (187.4)	1,155	189.5 (161.1)	396	3.9 (9.4)
o-TOL, ng/24 h	400	155.8 (295.0)	377	147.8 (83.57)	371	236.4 (801.7)	1,148	179.2 (491.4)	395	63.5 (128.3)

4-ABP, and *o*-TOL, statistical tests suggest an association of gender and biomarker levels—an effect that might be confounded by the higher cigarette consumption of male smokers (partial R^2 of factor gender ≤ 0.04). However, following adjustment for urinary creatinine excretion, in smokers, a gender effect was seen only for 2 biomarkers: total 1-OHP (females: 240 ± 406 ng/g_{ucreat}; males: 203 ± 135 ng/g_{ucreat}) and total NNAL (females: 158 ± 133 ng/g_{ucreat}; males: 132 ± 104 ng/g_{ucreat}) were actually higher in females. In nonsmokers, levels of all biomarkers except COHb were also generally higher in males, whereas COHb concentrations were comparable in both genders.

All biomarkers of exposure, with the exception of *o*-TOL, appeared to be associated with age, although the contribution of age to the total variation for the majority of biomarkers in smokers was below 4% (partial $R^2 < 0.04$). In general, biomarker of exposure levels of subjects 35 years or older had higher values than subjects aged 21 to 34 years, whereas levels of subjects aged 35 to

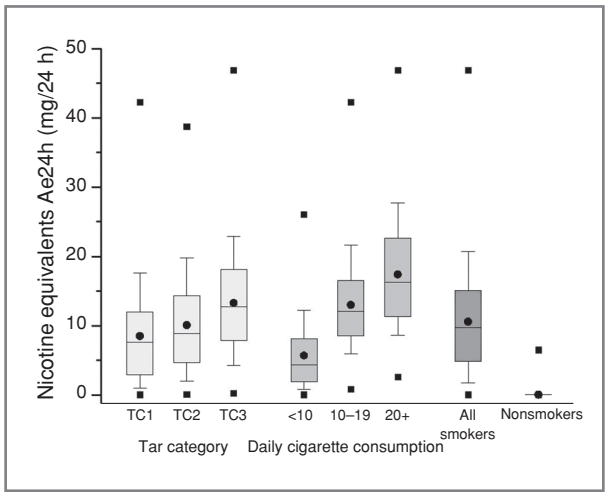

Figure 1. Urinary nicotine equivalents Ae24h (average of visits 2 and 3) in mg/24 h for smokers (by tar category, daily cigarette consumption, all smokers) and nonsmokers for the valid case population. Box and whisker plots: boxes represent 25th and 75th percentiles, whiskers represent 10th and 90th percentiles; ■ (squares) represent minimum and maximum in group/subgroup; ● (dots) represent mean in group/subgroup.

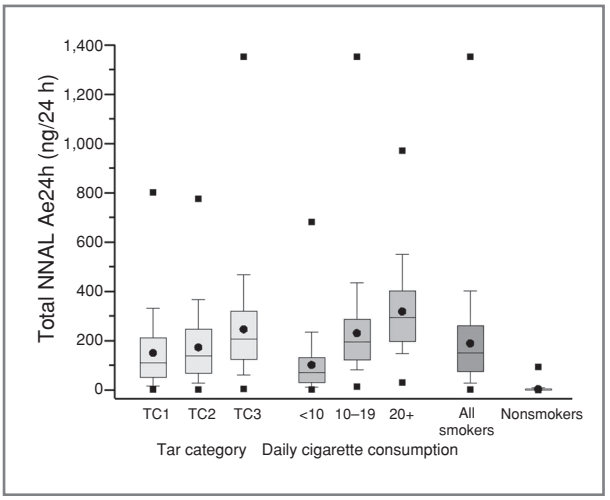

Figure 2. Urinary total NNAL Ae24h (average of visits 2 and 3) in ng/24 h for smokers (by tar category, daily cigarette consumption, all smokers) and nonsmokers for the valid case population. Box and whisker plots: boxes represent 25th and 75th percentiles, whiskers represent 10th and 90th percentiles; ■ (squares) represent minimum and maximum in group/subgroup; ● (dots) represent mean in group/subgroup.

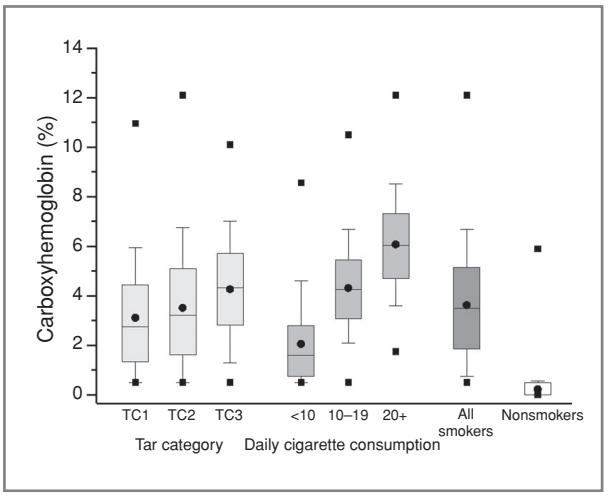

Figure 3. Carboxyhemoglobin saturation in blood at 6:00 PM ± 2 hours (average of visits 2 and 3) in % for smokers (by tar category, daily cigarette consumption, all smokers) and nonsmokers for the valid case population. Box and whisker plots: boxes represent 25th and 75th percentiles, whiskers represent 10th and 90th percentiles; ■ (squares) represent minimum and maximum in group/subgroup; ● (dots) represent mean in group/subgroup.

49 years and subjects 50 years or older were comparable. This small age effect might also be confounded by the higher cigarette consumption observed in older subjects.

Large differences between the countries were observed for COHb, PCOT, 2-NA, 3-HPMA, and total NNAL (Supplementary Table S3). Levels of PCOT and COHb were found to be lowest in Germany and highest in the United Kingdom, levels of 2-NA were lowest in Switzerland and highest in the United Kingdom, and levels of 3-HPMA and total NNAL were lowest in the United Kingdom. The contribution of the factor country to the total variation was marginal (partial $R^2 \leq 0.02$) for all biomarkers of exposure.

Only for PCOT, 2-NA, 3-HPMA, and total NNAL, an association of BMI and biomarker levels was found in smokers, with higher 3-HPMA and total NNAL values and lower PCOT and 2-NA values observed in subjects with higher BMI. Although the effect of BMI was significant for these 4 biomarkers, the increase of the overall R^2 of the ANOVA model upon inclusion of BMI was marginal (partial $R^2 \leq 0.01$) in all cases.

Cigarette filter analysis

The average cigarette butt length was 35.9 (±4.4) mm and was comparable in the 3 tar categories. The average nicotine content in the filters of all collected butts was 58.0 ± 25.0 μg/filter. The nicotine content in the filters in TC1 (52.6 ± 25.6 μg/filter) was slightly lower than in TC2 (60.7 ± 23.2 μg/filter) and TC3 (61.0 ± 25.3 μg/filter). In all tar categories, nicotine content in the filter appeared to slightly decrease with increased cigarette consumption and appeared to be lower during the weekend than on weekdays. Results from the cigarette filter analysis conducted are only indicative of the actual filter retention.

Safety

There were minimal differences in safety parameters between smokers and nonsmokers. In general, clinical laboratory parameters recorded at the screening visit were within normal ranges for the majority of subjects, the proportions of subjects having values of a particular

Table 5. Biomarkers of exposure by daily cigarette consumption (unadjusted values, valid case population)

Biomarker	Smokers									
	<10 cig/d (*N* = 467)		10–19 cig/d (*N* = 557)		≥20 cig/d (*N* = 135)		All smokers (*N* = 1,159)		Nonsmokers (*N* = 399)	
	n	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)
Concentration at 6:00 PM ± 2 h										
COHb, %	457	2.06 (1.63)	556	4.33 (1.76)	135	6.09 (1.88)	1,148	3.63 (2.21)	398	0.23 (0.40)
PCOT, ng/mL	458	119.7 (107.2)	557	253.1 (105.4)	135	340.3 (127.9)	1,150	210.2 (134.2)	398	2.23 (9.11)
Ae24h										
Neq, mg/24 h	464	5.73 (4.92)	556	13.05 (6.25)	135	17.46 (7.81)	1,155	10.62 (7.31)	396	0.05 (0.34)
2-NA, ng/24 h	464	9.47 (12.81)	557	22.03 (39.62)	135	26.73 (27.29)	1,156	17.54 (30.88)	395	1.79 (3.59)
4-ABP, ng/24 h	464	12.37 (11.06)	557	32.18 (106.93)	135	35.89 (17.43)	1,156	24.66 (75.44)	397	2.77 (11.79)
3-HPMA, mg/24 h	464	1.12 (0.90)	557	2.10 (1.20)	135	2.98 (1.27)	1,156	1.81 (1.26)	398	0.63 (0.80)
MHBMA, μg/24 h	464	1.82 (1.83)	557	3.93 (3.09)	135	5.55 (3.66)	1,156	3.27 (3.02)	398	0.30 (0.42)
S-PMA, μg/24 h	464	2.40 (2.16)	557	4.91 (3.27)	135	6.84 (4.08)	1,156	4.13 (3.36)	398	0.39 (0.27)
Total 1-OHP, ng/24 h	464	202.9 (175.9)	557	339.7 (457.4)	135	389.4 (218.4)	1,156	290.6 (352.2)	398	122.5 (100.5)
Total NNAL, ng/24 h	463	101.9 (105.9)	557	231.0 (159.1)	135	319.0 (171.2)	1,155	189.5 (161.1)	396	3.9 (9.4)
o-TOL, ng/24 h	464	121.0 (155.3)	552	216.1 (689.2)	132	229.9 (102.3)	1,148	179.2 (491.4)	395	63.5 (128.3)

parameter outside laboratory normal ranges (LNR) were comparable between smokers and nonsmokers. Greater differences in the proportion of subjects with values elevated outside LNR were found for mean corpuscular hemoglobin (MCH; smokers: $n = 264$, 21.6%; nonsmokers: $n = 49$, 12.0%), white blood cell (WBC) count (smokers: $n = 114$, 9.3%; nonsmokers: $n = 8$, 2.0%), and neutrophil count (smokers: $n = 85$, 7.0%; nonsmokers: $n = 6$, 1.5%).

Mean MCH values in smokers (31.3 ± 1.9 pg), without apparent differences between the 3 tar categories, and nonsmokers (30.8 ± 1.5 pg) were comparable (LNR: 27–32 pg). Mean WBC and neutrophil counts were observed to be higher in smokers (7.61 ± 2.11 G/L and 4.77 ± 1.76 G/L, respectively) than in nonsmokers (6.38 ± 1.56 G/L and 3.89 ± 1.29 G/L, respectively). For both parameters, a slight increase with increased tar yield was observed. Mean WBC counts in TC1, TC2, and TC3 were 7.44 ± 2.08 G/L, 7.45 ± 1.96 G/L, and 7.95 ± 2.25 G/L (LNR: 3.9–10.3 G/L); mean neutrophil counts in TC1, TC2, and TC3 were 4.61 ± 1.74 G/L, 4.67 ± 1.63 G/L, and 5.04 ± 1.89 G/L (LNR: 1.9–7.5 G/L). Levels of lactate dehydrogenase (LDH) and total bilirubin were lower in smokers than in nonsmokers. In smokers, the mean LDH level was 152.0 ± 31.1 U/L (TC1: 151.5 ± 30.5 U/L; TC2: 150.0 ± 33.0 U/L; TC3: 154.3 ± 29.7 U/L), with 306 subjects (25.0%) having values below the LNR (male: 135–225 U/L; female: 135–214 U/L) compared with a mean LDH level of 160.4 ± 29.5 U/L and 68 subjects (16.7%) with values below the LNR in nonsmokers. Total bilirubin mean levels were 7.74 ± 4.73 μmol/L for smokers (TC1: 7.28 ± 3.85 μmol/L; TC2: 8.01 ± 5.25 μmol/L; TC3: 7.95 ± 4.97 μmol/L) and 9.42 ± 6.60 μmol/L for nonsmokers; with 119 smokers (9.7%) and 21 nonsmokers (5.2%) having bilirubin levels below LNR (3.8–21.9 μmol/L). Mean high-density lipoprotein (HDL) levels of smokers (1.50 ± 0.38 mmol/L), particularly of smokers in the highest tar category TC3 (1.44 ± 0.38 mmol/L), were lower than in nonsmokers (1.54 ± 0.40 mmol/L). Mean HDL values in TC1 and TC2 were 1.55 ± 0.41 and 1.51 ± 0.35 mmol/L). Smokers (92.8%) and nonsmokers (94.1%) had normal HDL levels (LNR male: 0.93–2.35 mmol/L; female: 0.91–2.28 mmol/L).

A total of 592 smokers (48.4%) and 226 nonsmokers (55.4%) reported intercurrent illnesses during the study (including preexisting diseases). The most commonly reported disorders were nervous system (e.g., headache) followed by metabolism and nutrition disorders and musculoskeletal disorders.

The percentage of subjects taking concomitant medication during the study was comparable in nonsmokers ($n = 197$, 48.3%) and smokers ($n = 581$, 47.5%). Most commonly taken medications were sex hormones (mainly oral contraceptives) and medications for nervous system disorders.

There were no differences observed between smokers and nonsmokers with respect to vital signs and physical examinations.

Discussion

This was the largest multicenter study conducted in Europe so far to evaluate an extensive panel of biomarkers of exposure to potentially harmful cigarette smoke constituents and to investigate the association with smoking machine–determined tar yields. Other large studies have either been conducted in a single European population (8, 28) or been conducted in the United States (10, 29). The subjects in this study were well distributed over the study sites within each country, overall, and for each study group.

To substantiate the potential of a tobacco product to reduce the exposure to harmful cigarette smoke constituents, a reliable panel of biomarkers for assessing exposure in human smokers (13) and a baseline for evaluating changes in exposure of the general population following the introduction of new tobacco products are needed. This study has included biomarkers for 5 of the 9 toxicants (1,3-butadiene, acrolein, benzene, carbon monoxide, and NNK) recommended by the WHO for mandated lowering of exposure levels (13). For the remaining 4 smoke toxicants [acetaldehyde, benzo(*a*)pyrene, formaldehyde, and *N*′-nitrosonornicotine], suitable biomarkers of exposure and/or analytic methods were not available at the time of study conduct.

The present study provides data for biomarker of exposure levels in adult smokers and nonsmokers in 3 European countries; smokers were found to have significantly higher levels of all the investigated biomarkers of exposure than in nonsmokers. For all but 3 biomarkers (total 1-OHP, 4-ABP, and *o*-TOL), large differences in biomarker levels were observed between smokers and nonsmokers in the TC1 and TC3 groups. Only for total NNAL were large differences found between TC1 and TC2 and between TC2 and TC3. For COHb, Neq, 2-NA, and 3-HPMA, differences between TC1 and TC2 and for PCOT, a difference between TC2 and TC3 were observed. These results are not totally surprising, as total NNAL is considered to be tobacco specific, whereas for the other biomarkers of exposure, except Neq and PCOT, multiple sources of non–tobacco-related exposure occur in both smokers and nonsmokers. However, the absence of consistent differences for both Neq and PCOT between TC1 and TC2, and between TC2 and TC3, cannot be explained.

All biomarkers of exposure were strongly associated with the daily number of cigarettes smoked. The highest levels of all biomarkers of exposure were observed in smokers smoking 20 or more cig/d, and the lowest levels were in smokers of less than 10 cig/d. Daily cigarette consumption was found to be a stronger predictor of biomarkers of exposure levels than the ISO tar and nicotine yields. These results are consistent with the results of another study conducted in a German population of 274 smokers and 100 nonsmokers, in which biomarkers of exposure measured in urine, blood, and saliva had only a weak association with the machine-derived ISO tar and nicotine yields, and levels of biomarkers of

exposure for smokers of low ISO tar yield cigarettes were not as low as might be predicted by the ISO tar and nicotine yields (8). Adjustment of biomarkers of exposure levels for the number of cigarettes smoked resulted in an inverse relationship of biomarker levels and consumption, with the lowest per-cigarette levels observed in the subjects with the highest consumption. It appears that an increased daily consumption corresponds to a reduced consumption per cigarette.

It is interesting to note here that levels of all biomarkers of exposure except *o*-TOL were associated with age. However, this age effect may more likely be explained by the observed increased cigarette consumption in older subjects. The gender effect seen for all biomarkers of exposure except COHb, 4-ABP, and *o*-TOL may also be more likely explained by the observed higher cigarette consumption of males. The reanalysis examining the effect of BMI suggested that BMI was a significant factor ($P < 0.05$) for PCOT, 2-NA, 3-HPMA, and total NNAL but not for other biomarkers of exposure. However, even in these 4 cases, the additional contribution of BMI to the overall R^2 was marginal (partial $R^2 \leq 0.01$). In addition, in a model including age, gender, and BMI, BMI cannot be considered to be an independent factor. Similar results, that is, significant effects of BMI on biomarker levels with a similarly small contribution to the overall R^2, have previously been reported for PCOT and total NNAL (30, 31).

In general, the model including the factors tar category, daily cigarette consumption, age, gender, and country showed a reasonable fit for COHb, PCOT, Neq, and 3-HPMA (with overall R^2 ranging from 0.3436 for 3-HPMA to 0.4696 for COHb), a weak fit for MHBMA, *S*-PMA, and total NNAL ($0.2240 \leq R^2 \leq 0.2936$) but only a poor fit for 2-NA, 4-ABP, total 1-OHP, and *o*-TOL ($R^2 \leq 0.0867$). Including BMI as a fixed factor in the model did not improve the fit substantially for any of the biomarkers of exposure. Although demographic variables (age, gender, country) were statistically significant factors in most models and regardless of the fit of the model, the contribution of these factors to the overall R^2 was rather small in all models (partial $R^2 \leq 0.04$ for all demographic variables). The same applied to the tar category: although this was a statistically significant factor in all models except for 4-ABP, total 1-OHP, and *o*-TOL, the partial R^2 of the factor tar category was never larger than 0.02 (i.e., not more than 2% of the overall variation was explained by the tar category). In contrast, the daily cigarette consumption always contributed the most to the explanation of the variation. For the 7 biomarkers of exposure for which the model showed a weak or reasonable fit, the partial R^2 for that factor ranged from about 0.13 to 0.17 (3-HPMA, MHBMA, *S*-PMA, total NNAL) to about 0.24 (PCOT, Neq) and 0.30 for COHb.

All 4 study groups were found to have high variability in biomarkers of exposure levels, which is consistent with previous reports and not surprising due to the ambulatory nature of the study (10). In a study evaluating the relationship of several biomarkers of exposure in a total of 400 subjects from 2 different clinical trials, there was considerable variability in exposure to cigarette smoke constituents, especially among the highest tar yield smokers (29). Each of the biomarker levels appeared to plateau around 25 to 35 cig/d. However, that study did not account for tar yield and so the present study may provide some additional insight into the relationship between tar yield and daily cigarette consumption. Individual smoking patterns, such as depth of inhalation, as well as the limitations of self-reporting of daily cigarette consumption, can also increase variability biomarkers of exposure levels (9, 29, 32). Still, the differences seen between smokers and nonsmokers and the observed dose effect (characterized by daily consumption and tar yield) support the use of the biomarkers of exposure measured in this study in the assessment of tobacco products (9, 33).

Results from the cigarette filter analysis are only indicative of filter retention and cannot be extrapolated to actual exposure. This would require a calibration by cigarette brand, which could not conducted, as necessary information for calibration was not available for all cigarette brands. Differences in cigarette design could affect the retention of nicotine in the filter.

Over the course of the study, 18 subjects switched cigarette brands at least once and about half of those switched to brands of a different tar yield. The allocation to a specific tar category for those subjects was done on the most frequent cigarette brand smoked over the full study. The effect of brand switching during the study on the study results was not further analyzed; however, this is not expected to have affected the results.

The present study was an observational study investigating the effect of the ISO tar yield of a smoker's cigarette brand and other potentially confounding factors on the exposure of smokers to selected cigarette smoke constituents. The current WHO recommendation is to assess tar and nicotine yields based on the Health Canada Intense (HCI) smoking regimen, and it would be of interest to investigate the relationship between biomarkers of exposure levels and yields determined according to the HCI regimen. However, the HCI regimen to measure machine-determined tar, nicotine, and carbon monoxide yields is not used in Europe for labeling of cigarette packs and this information was not available. Thus, the reported ISO yields, as found on the cigarette packaging, were used for grouping of cigarette products and smokers in the current study. In the statistical analysis, the tar category effect was tested adjusting for the factors of daily cigarette consumption, age, gender, and country. However, a comprehensive analysis of these factors, their interactions, and other potential confounders was not conducted. Because of the exploratory nature of the study and because no multiplicity adjustments were done, *P* values should be interpreted cautiously.

In conclusion, large differences were observed between smokers and nonsmokers for all biomarkers

measured in this study. For the majority of biomarkers, there is an increase in exposure with increasing ISO tar yield and daily cigarette consumption. However, daily cigarette consumption was found to be a much stronger predictor of exposure than the ISO tar yield, although apparently the increased daily cigarette consumption corresponded to a reduced consumption per cigarette.

The results of this study support the use of these biomarkers in future studies to assess exposure in the assessment of tobacco products. As this was an exploratory study, the observed differences among the tar groups should be interpreted carefully and must not be interpreted as a reduction in exposure or risk for smokers of low-tar cigarettes.

Disclosure of Potential Conflicts of Interest

D. Lindner and C. Martin Leroy are former employees of Philip Morris Products S.A.; A. Tricker is a current employee of Philip Morris Products S.A. No potential conflicts of interest were disclosed.

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