Comparison of Exposure to Selected Cigarette Smoke Constituents of Adult Smokers and Non-smokers in a European Multi-centre, Observational Study

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INTRODUCTION AND OBJECTIVES

There are over 4000 identified compounds in cigarette smoke to which a smoker is exposed. Due to the large variation in individual smoking behaviour, machine-derived yields of cigarette smoke constituents (CSC) are not an accurate representation of the uptake of those CSCs by smokers. A better indication of CSC uptake can be obtained by measuring biomarkers of exposure (BoExp) in body fluids. BoExp represent an internal dose of tobacco smoke and provide information regarding short-term or long-term tobacco exposure. [1]

A European, multi-centre, observational study was performed to determine the exposure of adult smokers to selected potentially harmful CSCs. Non-smokers were also included to provide information about background levels of these BoExp.

METHODS

STUDY CONDUCT AND ASSESSMENTS

This study was conducted in three countries (United Kingdom [UK], Germany, and Switzerland) according to the principles of Good Clinical Practice [2]. Healthy adults, either smokers who smoked cigarettes (for at least 1 year) or non-smokers (for at least 1 year) were enrolled. Smokers were stratified according to the ISO[a] tar level of their usual cigarette brand (≤ 4.9, 5.0-7.9, and ≥ 8.0 mg ISO tar yield).

Blood samples (at 18:00 ± 2h) and urine samples (24h urine) were taken at two visits (within 1 week) and analysed for BoExp for ten CSCs (see Table 1) using validated methods.

Table 1: Smoke Constituents and Related Biomarkers of Exposure Measured

Smoke constituent	Biomarker	Matrix	Analytical Method
Nicotine	Nicotine equivalents (nicotine + 5 major metabolites,[Neq])	Urine	HPLC-MS/MS
	Cotinine (COT-P)	Plasma	HPLC-MS/MS
Carbon monoxide (CO)	Carboxyhaemoglobin (COHb)	Blood	Oximetry
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronides (Total NNAL)	Urine	HPLC-MS/MS
Benzene	S-phenylmercapturic acid (S-PMA)	Urine	HPLC-MS/MS
Acrolein	3-hydroxypropyl-mercapturic acid (3-HPMA)	Urine	HPLC-MS/MS
1,3-butadiene	Monohydroxybutenyl mercapturic acid (MHBMA)	Urine	HPLC-MS/MS
Pyrene	1-hydroxypyrene and its glucuronide and sulphate conjugates (Total 1-OHP)	Urine	HPLC-MS/MS
o-toluidine	o-toluidine	Urine	HPLC-MS/MS
4-aminobiphenyl (4-ABP)	4-ABP/erythrocytes (4-ABP/ery) 4-ABP	Blood Urine	HPLC-MS/MS
2-aminonaphthalene (2-NA)	2-NA	Urine	HPLC-MS/MS

STATISTICAL METHODS

Analysis of BoExp levels was based on the averages of the two study visits of blood/plasma concentration (COT-P, COHb, 4-ABP/ery) or 24h urinary excretion ([QE24h] for urinary BoExp).

BoExp levels in smokers and non-smokers were compared using the Wilcoxon rank sum test. Differences between the BoExp levels for the three smoking groups were tested using analysis of variance with adjustment for country, gender, age, and daily cigarette consumption, followed by pair-wise comparisons.

[a] ISO = International Organization for Standardization

RESULTS

STUDY POPULATION

A total of 1250 smokers and 417 non-smokers were enrolled and data from 1159 smokers and 399 non-smokers were evaluated (Table 2). Demographic data and daily cigarette consumption during the study is summarised in Table 3.

Table 2: Study Population (Analysis Population)

Study Group	UK	Germany	Switzerland	Total
Smokers	393	510	256	1159
Low tar (≤ 4.9 mg)	144	170	88	402
Medium tar (5.0-7.9 mg)	128	172	79	379
High tar	121	168	89	378
Non-Smokers (≥ 8.0 mg)	139	169	91	399
Total	532	679	347	1558

Table 3: Demographic Data and Daily Cigarette Consumption

	Smokers				Non-Smokers N=399
	Low Tar N=402	Medium Tar N=379	High Tar N=378	All Smokers N=1159	
Gender					
Male n (%)	136 (33.8)	136 (35.9)	189 (50.0)	461 (39.8)	192 (48.1)
Female n (%)	266 (66.2)	243 (64.1)	189 (50.0)	698 (60.2)	207 (51.9)
Age (years)					
Mean (SD)	36.0 (11.7)	34.5 (11.2)	37.0 (11.8)	35.8 (11.6)	44.0 (15.8)
Min-Max	21-78	21-72	21-72	21-78	21-85
Daily Cigarette Consumption (cig/d)					
Mean (SD)	10.5 (5.8)	11.1 (6.4)	13.8 (6.2)	11.8 (6.3)	---
Min-Max	1-37	1-39	1-35	1-39	---
<10 cig/d (n)	190	179	95	464	---
10-19 cig/d (n)	181	157	220	558	---
20-30 cig/d (n)	30	39	57	126	---
>30 cig/d (n)	1	4	6	11	---

BIOMARKERS OF EXPOSURE

Smokers were found to have significantly higher levels of all BoExp compared to non-smokers. The largest differences between smokers and non-smokers were reported for plasma cotinine, COHb, nicotine equivalents, and total NNAL.

High variability in BoExp levels was observed, particularly in the smoking groups. For all BoExp an association was found between BoExp levels and both daily cigarette consumption and tar levels. In general, higher BoExp levels were observed in smokers of higher tar yield cigarettes (see sample figures). However, BoExp levels were more closely associated with daily cigarette consumption (Table 4).

Age, gender, and country were also found to be confounding factors for the majority of the BoExp measured. The same trends were observed in the urinary BoExp when adjusted by urine creatinine.

Table 4: Mean (SD) Levels of Biomarker of Exposure

| Biomarker	Smokers				Non-Smokers N=399
	<10 cig/d N=464	10-19 cig/d N=558	20-30 cig/d N=126	All Smokers N=1159	
COT-P (ng/ml)	119.6 (107.4)	252.1 (105.6)	333.1 (117.3)	210.2 (134.2)	2.2 (9.1)
COHb (%)	2.1 (1.6)	4.3 (1.8)	6.0 (1.8)	3.6 (2.2)	0.2 (0.4)
Neq (mg/24h)	5.7 (4.9)	13.0 (6.3)	17.0 (8.0)	10.6 (7.3)	<0.1 (0.1)
Total NNAL (ng/24h)	102.2 (106.1)	230.0 (159.2)	309.5 (167.2)	189.5 (161.1)	3.9 (9.4)
S-PMA (µg/24h)	2.38 (2.16)	4.88 (3.20)	6.78 (4.30)	4.13 (3.36)	0.39 (0.27)
3-HPMA (mg/24h)	1.12 (0.90)	2.10 (1.20)	2.88 (1.23)	1.81 (1.26)	0.63 (0.80)
MHBMA (µg/24h)	1.82 (1.84)	3.91 (3.06)	5.43 (3.69)	3.27 (3.02)	0.30 (0.42)
Total 1-OHP (ng/24h)	202.7 (176.4)	338.3 (456.4)	385.7 (228.6)	290.6 (352.2)	122.5 (100.5)
o-toluidine (ng/24h)	114.9 (77.7)	220.4 (698.9)	219.8 (96.7)	179.2 (491.4)	63.5 (128.3)
4-ABP urine (ng/24h)	12.3 (11.1)	32.1 (106.8)	34.7 (17.3)	24.7 (75.4)	2.8 (11.8)
4-ABP/ery (pg/ml)	0.015 (0.013)	0.026 (0.014)	0.035 (0.016)	0.023 (0.015)	0.004 (0.003)
2-NA (ng/24h)	9.5 (12.8)	21.9 (39.5)	26.2 (27.4)	17.5 (30.9)	1.8 (3.6)

Fig. 1: Nicotine equivalents QE24h
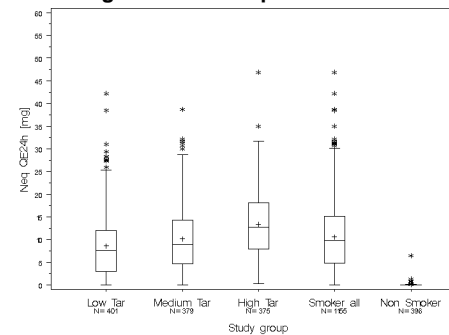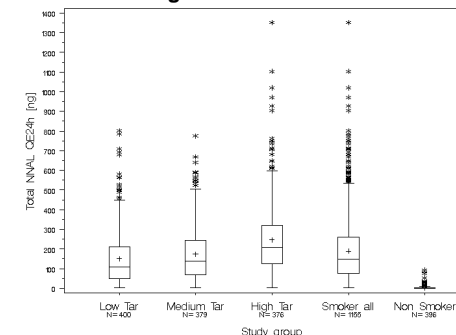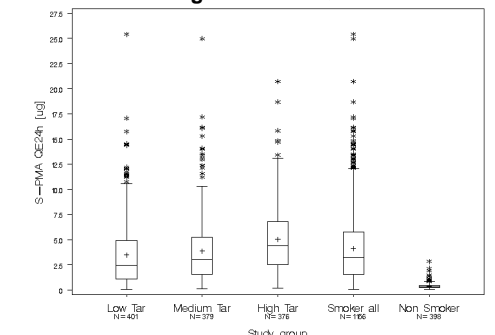

Fig. 2: Total NNAL QE24h

Fig. 3: S-PMA QE24h

DISCUSSION AND CONCLUSIONS

This is one of the largest multi-centre studies performed to evaluate an extensive panel of BoExp to potentially harmful CSCs. The study provides a snapshot of BoExp levels in adult smokers and non-smokers in three European countries. The results can be used as a baseline to evaluate how changes in legislation and introduction of new products could influence CSC uptake in the general population. It demonstrates that smokers have significantly higher levels of all the BoExp investigated than non-smokers. The differences seen between smokers and non-smokers confirm that the BoExp selected would be suitable to assess CSC uptake in early assessment of potential reduced-risk tobacco products.

Smokers were found to have a large variation in BoExp levels. These levels are more closely associated with daily cigarette consumption than with ISO nicotine or tar yields. This is in line with another study conducted in a German population [3]. The results from this study must not be taken as an indication that the risk of smoking low tar cigarettes is any less than that of smoking cigarettes with a higher tar yield.

REFERENCES

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