Environmental Tobacco Smoke and Urinary Cotinine in a Group of Adolescent.

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16 October 2023

INVOLUNTARY EXPOSURE TO TOBACCO SMOKE IN ADOLESCENTS. URINARY COTININE AND ENVIRONMENTAL FACTORS.

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ABSTRACT. The relationship between environmental tobacco smoke exposure and urinary cotinine measured in urine of 434 fourteen years old schoolchildren was studied. In order to estimate the independent contribution of the physiological and environmental variables to cotinine concentrations, a multiple regression analysis of the log transformed cotinine levels was carried out ($R^2 = 0.21$, $p < 0.0001$).
The present findings confirm that the passive exposure to tobacco smoke is complex phenomenon not evaluable with single environmental contributions. Furthermore, it is a phenomenon directly linked to the number of cohabitants who smoke, in particular to the smoking habit of mother ($\beta = 5.135$, $p = 0.0397$) or mother and other cohabitants ($\beta = 8.201$, $p = 0.0020$) and to the number of cigarettes smoked by all the cohabitants ($\beta = 0.217$, $p = 0.0008$). Passive smoke exposure of adolescents is a preventable risk by providing a higher quality home environment and, above all, suggesting that parents avoid smoking at home.

INTRODUCTION

Passive smoke is considered an important risk factor for several diseases. Passive smokers are unintentionally exposed to several toxic chemicals[1,2] and, consequently, to hazards for lung cancer, heart, circulatory and respiratory diseases[3,4,5,6,7,8]. Markers of exposure have been observed in biological fluids of passive smokers, although less than in active smokers. These include metabolites of tobacco smoke[9,10], activation of enzymatic systems metabolising carcinogens[11] and an increase of urinary mutagens[12,13].

Cotinine, a nicotine metabolite, is measurable also in passive smokers and is a highly specific dose-marker of tobacco smoke exposure. Cotinine has a half-life of 20 hours[14] and it can be measured in urine which facilitates its use in epidemiological studies regarding general population and, in particular, children and adolescents. Some epidemiological studies have been carried out in the last years relating cotinine and degrees of passive exposure to tobacco smoke in different populations: 6-8 weeks old newborns[15], children[6,7,16], adult passive smokers or adult non-smokers[17,18,19] but nobody has considered a relevant number of adolescents. This age is very important considering: the close relations with smoke habit of parents, the light social life (school and/or sport) and, therefore, the numerous possibilities of passive exposure to environmental tobacco smoke, the emulation of smoking habits, and risk factors for cancer and mortality[20,21].

Thus, in this epidemiological study we examined the relationship between environmental tobacco smoke exposure and a biological marker measured in urine of 434 fourteen years old schoolchildren: cotinine and cotinine/creatinine ratio (CCR).

Moreover, the validation of exposure data arising from an administered questionnaire has been carried out, and some physiological characteristics of the subjects, as well as some environmental aspects, have been considered as potentially confounding factors. The above mentioned relationship has been evaluated considering urinary thiocyanate and measuring the environmental air nicotine exposure by means of a diffusive (passive) personal sampler fasten to the collar of each subject. The effectiveness of this environmental-objective marker could allow an easier approach to epidemiological studies concerning passive exposure to tobacco smoke.

MATERIALS AND METHODS

1. Epidemiological sample. The epidemiological sample construction considered three districts of Turin city (North-Western Italy), the first in the centre, the second on the semi-outskirts, and the third on the outskirts of the city. In each district, all the high schools were identified and, using a random method, one school was chosen. Subsequently, all the 434 schoolchildren (males and females) attending the first year of the three schools were involved in the study. One school at a time was considered and the whole sampling work lasted four months, from December 1991 to March 1992.

2. Data collection and analysis. For each subject the procedure included: a) administration of a *questionnaire*, b) collection of an *urine sample*, c) use of a *personal passive sampler,* and d) *measurement of lung function*.

Data collection was carried out from Monday to Friday each working week and the procedure lasted two days for each subject. During the first of the two days, the sampler and an urine container were supplied, between 8 and 9 a.m. The second day, sampler and container were returned at the same hour, the respiratory functionality was measured, and, immediately after, the questionnaire was administered.

a) Questionnaire. 90 questions were addressed to all the subjects by one interviewer. The questions concerned the following items: • individual: sex, age, residence, smoking habit, n° of hours spent at home, at school, outdoor, • familiar: composition, social characteristics, smoking habits of each component, • environmental: square meters of the apartment, number of rooms, age of building, degrees of ventilation.

The questionnaire was re-administered to 80 subjects after one month by the same interviewer. The double answers underline a "K" (index of Cohen) = 0.93 (range 0.89-0.95) where: K = (observed agreements-expected agreements) x (1-expected agreements)$^{-1}$

b) Urine sample. The urine containers were filled with the first urine of the morning on the second day of the procedure. Samples ($\approx$100 ml) were used for cotinine, creatinine, and thiocyanate measurements. Creatinine and thiocyanate were measured immediately after collection, while cotinine not later than one month (stored at -80°C).

• Cotinine analysis[22,23]. <u>Extraction</u>. 25 ml of urine, 1 ml of NaOH 5M, 8.5 gr of NaCl and 5 ml of chloroform were added in a "centrifuge tube" (50 ml) and shacked for 5 min and, subsequently, centrifuged (1000 rpm) for 10 min. 2 ml of chloroformic layer were withdrawn, dried, and re-dissolved with 200 µl of chloroform. <u>Analysis.</u> It was carried out using a Gas-Chromatograph equipped with a flame ionisation-nitrogen selective detector (NPD) with a programmed temperature. <u>Quality control.</u> The external quality control relative to this G.C. technique was carried out re-analysing 20 times a cotinine standard solution added to a pool of non-smokers urine. The results show a Coefficient of Variation = 4.3%, a detection limit below 1 ng/ml, and recovery = 100%. The internal quality control was carried out using pyridine and the obtained results have been reported in a previous paper[24].

• Thiocyanate analysis. Measurement of thiocyanate were completed by the spectropho-tometrical method originally described by[25]; the optical absorption was determined at 520 nm.

c) Personal air samples were collected for 24 hours by means of a passive (diffusive) sampler specific for airborne nicotine measurements[26] and fasten to the collar of each student. <u>Sampler.</u> The sampler was equipped, immediately before use, with a fiber glass filter/teflon coated ($\varnothing$ 37 mm). This last was coated with 500 µl of a 4% sodium bisulphite and 5% ethanol solution, dried and sealed into a teflon pocket until use. <u>Extraction.</u> The used filter, 2 ml of H_2O, and 100 µl of ethanol were put in a centrifuge tube (10 ml) and shacked for 1 min; 2 ml of NaOH 10 N were added and the centrifuge tube was re-shacked for 1 min. Finally, 250 µl of eptane were added, the tube shacked for 2 min. and 2 µl of eptane layer introduced in the G.C. Quality control data related to this measurement were reported in a previous paper[24]. <u>Analysis.</u> It was carried out using the same equipment used for the cotinine analysis.

d) Lung function. Maximal expiratory flow-volume curves were obtained with the subjects in standing position, with nose clips, breathing through a Collins Stead Wells computerised spirometer. The instrument was calibrated daily with a 3 liters syringe. All graphs were printed and the measures retained until three curves, varying no more than 5%, were obtained. Values were corrected to (BTPS). The best forced vital capacity (FVC) and forced expiratory volume in 1 sec. (FEV_1) together with forced expiratory flows at 50% (MEF_{50}) and 25% (MEF_{25}) from the curve with the best sum $FVC + FEV_1$ were selected[27]. The body mass index (B.M.I.)

- weight/height^2 - was calculated with the aim to consider this individual condition as a possible confounding factor involved in the composition of the epidemiological sample.

3. <u>Statistical analysis.</u> Distribution of urinary cotinine values was skewed [Shapiro-Wilk test[28]: W = 0.9154; p = 0.0001]. Thus, Spearman rank order correlation coefficients were calculated for continuous variables. Determinants of the levels of cotinine were examined using multiple linear regression analysis. A logarithmic transformation of the cotinine concentrations was performed and results were converted in an untrasformed scale for presentation. All analysis were executed with S.A.S. Packages[29].

RESULTS

Of the 434 students analysed on the whole, 387 were no-smokers and they declared a complete passive exposure history; 287 were females (14.32 ± 0.60 years old) and 100 were males (14.13 ± 0.37 years old). Measurement of body mass indexes did not show differences between the two groups of students: 2.02 ± 0.30 in females and 2.02 ±0.33 in males.

Considering the answers to questionnaires, four groups of passive exposure to tobacco smoke were identified: not exposed, exposed to others cohabitants but not mother, exposed to mother, and exposed to mother and others cohabitants. According to sex and to these four types of exposure, **table 1** shows the urinary concentrations of cotinine, CCR, thiocyanate, and the nicotine levels measured by personal air samplers.

Means and medians of cotinine data are directly proportional to the trend of exposure classes. This is confirmed in females, while in males the class exposed to mother shows higher levels of cotinine but also a small number of components (6 subjects). Females show, in general, cotinine levels higher than males. Similar observations are obtained considering CCR. On the contrary, thiocyanate do not show correlations with the degree of passive exposure to tobacco smoke, as well as nicotine. In this last case, the medians are stable in the 4 classes considered, either on the whole or disaggregated by sex.

Figure 1 shows medians and interval of confidence (IC) 95%, of cotinine, CCR, thiocyanate, and nicotine, considering passive exposure degrees.

Others variables examined as factors involved in the cotinine and CCR urinary concentrations are: the total number of cigarettes daily smoked by parents, the body mass indexes (B.M.I.), the blood volume (B.V.) calculated using a Ciba-Geigy nomogram from age, body mass, and sex[30], some measurements of respiratory functionality (FEV$_1$, FVC, MEF$_{25}$, MEF$_{50}$), the age of buildings and square meters/person (as indirect environmental marker).

Table 2 shows the Spearmann correlation coefficients between these variables and urinary concentrations of cotinine and CCR. These last are positively and significatively correlated with the passive exposure to tobacco smoke quantitatively measured as number of cigarettes smoked by mother or by father. Furthermore, cotinine is inversely correlated with the year of construction of buildings and with m^2/person, and the two biological markers are inversely, but not always significatively, correlated with physiological variables (B.V., FEV$_1$, FVC, MEF$_{25}$, MEF$_{50}$).

In order to estimate the indipendent contribution of the variables considered to cotinine concentrations, a multiple regression analysis of the log transformed cotinine values was carried out. The following variables were considered as *indipendent variables*: sex (0 = female, 1 = male), the classes of passive exposure to tobacco smoke (0 = not exposed -reference category- , 1 = exposed to others but not to mother, 2 = exposed to mother, 3 = exposed to mother and others), and, as continuous variables, the total number of cigarettes smoked by mother, father and others, the year of construction of building, the m^2/person, the physiological variables, the B.V., and the B.M.I.

Table 3 shows that the log-urinary cotinine, adjusted by blood volume and lung function, is predicted by exposure classes, exposure to cigarettes smoked but not by sex, year of construction of buildings and m^2/person.

Figure 2 shows the urinary cotinine average estimated according to multiple regression coefficient of exposure classes (reference category = not exposed) and exposure to tobacco passively smoked (5, 10 and 15 cigarettes/die) corrected by the other variables considered in the regression model.

DISCUSSION

Our results identify a direct relationship between levels of passive exposure to tobacco smoke described by questionnaire and urinary cotinine, taken as dose-marker. This observation allows to consider the answers to the questionnaire as a variable describing the tobacco smoke exposure.

CCR trend does not show differences from cotinine, underlining the lack of influence of daily urine volume. Thiocyanate confirm its total inadequacy to discriminate the degree of exposure to passive smoke. The relationship between personal air nicotine exposure data and the levels of passive exposure to tobacco smoke does not prove statistically significant. This type of measurement is probably not sensitive enough to detect these low environmental tobacco smoke pollution levels. Thus, nicotine measured using the passive samplers does not seem to represent an alternative to biological markers in the present epidemiological study.

The present sample, observed for the first time considering urinary cotinine in such a large number of subjects, represents a particular group of people (14 years old students) having a life-style still strictly bound to home environment and, therefore, to the smoke habit of parents. Nevertheless, the questionnaire has provided informations showing also a limited social life that, excluding the hours spent at home and school (about 21-22 hours), is composed of shopping or sport activities. This behaviour can justify, on one hand, a not complete absence of exposure to tobacco smoke (cotinine median of the 156 "no smokers" = 10.6 ng/ml) and, on the other hand, a little active exposure (from 1 cig./week to 5 cig./day) for 47 students. The levels of urinary cotinine in these light smokers are, on average, around 50 ng/ml, lower than the cut-off[31] discriminating the smoking status of adults (100 ng/ml).

Results showed in table 2 underline a direct correlation between urinary cotinine and the number of cigarettes smoked by mother or father. Furthermore, the inverse correlation with lung function data shows the influence of exposure to tobacco smoke on respiratory functionality. This effect is however not remarkable considering that the exposure is a passive exposure regarding adolescents (regression coefficient from 0.03 to 0.16). Finally, the negative coefficients showed by environmental parameters suggest to consider these factors as influencing the internal dose levels of cotinine.

The difference between sex in cotinine and CCR distribution in table 1 was analysed using multivariate regression. During the foreward procedure the "sex" variable was introduced and showed a significant contribution inside the model. However, the subsequent introduction of the variable "blood volume", calculated considering age, body mass, and sex, determined the disappearance of significativity of sex and of blood volume itself. This results can explain that the variable "sex" is not a factor determining the cotinine and CCR distribution data but that it hides other factors.

In general, the present findings confirm the passive smoking exposure as a phenomenon directly linked to the number of cohabitants who smoke, in particular to the smoking habit of mother (qualitative relation) and to the total number of cigarettes smoked by cohabitants (quantitative relation) independently by who smokes at home.

The involuntary smokers show urinary cotinine levels ranging between 10.6 and 24.8 ng/ml, demonstrating a similar level of exposure inside such an homogeneous population for age and life style. Comparing this close range of cotinine levels to the results of other studies concerning other classes of age[6,7,9,15,16,17,18,19] it is possible to consider the present finding as original and as an useful contribution to the knowledge in this scientific field.

In conclusion, the passive exposure to tobacco smoke is a complex phenomenon not only evaluable with single environmental contributions. So, the reported results allow to consider the involuntary smoke exposure of adolescent as a preventable risk factor providing higher quality of home environment (m^2/person, ventilation, etc.) and, above all, suggesting parents to avoid smoking at home in presence of children.

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Table 2. Spearmann correlations between urinary concentrations of cotinine and CCR and some environmental and physiological variables.

	COTININE		CCR	
	r	p	r	p
MATERNAL SMOKING (n. of cig.)	0.39	0.0001	0.38	0.0001
PATERNAL SMOKING (n. of cig.)	0.26	0.0001	0.28	0.0001
B.M.I.	0.03	0.5096	0.01	0.8407
B.V.	-0.12	0.0140	-0.09	0.0827
FEV1	-0.01	0.7899	-0.11	0.0376
FVC	-0.03	0.5674	-0.09	0.0763
MEF25	-0.03	0.5161	-0.12	0.0175
MEF50	-0.08	0.1218	-0.16	0.0021
YEAR OF CONSTR. OF BUILDINGS	-0.11	0.0356	-0.09	0.0640
m^2/person	-0.11	0.0276	-0.11	0.0283

Table 3. Multiple regression analysis predicting log-urinary cotinine (R^2=0.21; F=7.625; p < 0.0001).

VARIABLES	CATEGORIES	β	p
sex	0 = females		
	1 = males	-6.405	0.2109
exposures classes	0= not exposed	0*	
	1= exposed to others, not mother	0.043	0.9823
	2= exposed only to mother	5.135	0.0397
	3= exposed to mother and others	8.201	0.0020
exposure to tobacco smoke	number of cigarettes	0.217	0.0008
year of construction of buildings	year	-0.079	0.0546
m^2/person		0.001	0.9892

Adjusted by blood volume and lung function data. * reference category.

Table 1. Cotinine, CCR and thiocyanate urinary concentrations and nicotine data by sex and degree of passive exposure to tobacco smoke.

	A	B	C	D
COTININE				
ALL SUBJECTS (n)	156	118	39	74
median *(Q1-Q3)*	**10.6** *(6.5-16.1)*	**13.7** *(7.1-23.7)*	**18.0** *(11.9-27.2)*	**24.8** *(15.7-33.4)*
mean *(SD)*	**13.4** *(10.7)*	**17.6** *(13.2)*	**22.0** *(13.8)*	**28.4** *(17.8)*
FEMALES (n)	112	88	33	54
median *(Q1-Q3)*	**11.5** *(6.7-18.3)*	**14.1** *(7.6-24.6)*	**18.0** *(11.9-25.0)*	**26.9** *(18.5-36.5)*
mean *(SD)*	**14.6** *(11.90)*	**18.4** *(13.6)*	**21.0** *(13.0)*	**31.8** *(19.4)*
MALES (n)	44	30	6	20
median *(Q1-Q3)*	**9.2** *(6.1-13.9)*	**12.8** *(6.5-20.3)*	**23.4** *(13.9-41.4)*	**16.4** *(14.1-25.1)*
mean *(SD)*	**10.5** *(6.2)*	**15.3** *(11.9)*	**27.5** *(17.9)*	**19.3** *(6.8)*
CCR				
ALL SUBJECTS (n)	156	118	39	74
median *(Q1-Q3)*	**7.1** *(4.8-10.5)*	**9.5** *(5.6-15.4)*	**12.42** *(8.4-18.9)*	**16.3** *(10.1-21.7)*
mean *(SD)*	**8.8** *(6.6)*	**11.5** *(7.8)*	**13.7** *(7.0)*	**18.6** *(12.2)*
FEMALES (n)	112	88	33	54
median *(Q1-Q3)*	**7.3** *(4.8-11.4)*	**10.1** *(6.2-15.9)*	**12.2** *(8.9-18.2)*	**16.6** *(11.0-25.3)*
mean *(SD)*	**9.3** *(7.1)*	**12.2** *(8.2)*	**13.4** *(6.8)*	**20.3** *(13.4)*
MALES (n)	44	30	6	20
median *(Q1-Q3)*	**6.8** *(4.7-8.1)*	**7.8** *(5.2-14.3)*	**14.2** *(7.6-21.4)*	**12.3** *(9.1-18.5)*
mean *(SD)*	**7.4** *(5.0)*	**9.7** *(6.4)*	**15.2** *(8.7)*	**13.9** *(6.5)*
THIOCYANATE				
ALL SUBJECTS (n)	156	118	39	74
median *(Q1-Q3)*	**92.6** *(68.9-116.6)*	**89.9** *(164.1-110.3)*	**94.7** *(80.0-110.0)*	**97.3** *(75.9-122.7)*
mean *(SD)*	**96.1** *(36.8)*	**93.0** *(51.5)*	**96.9** *(28.5)*	**98.7** *(34.3)*
FEMALES (n)	112	88	33	54
median *(Q1-Q3)*	**93.5** *(70.0-116.6)*	**92.4** *(65.7-110.6)*	**93.1** *(80.0-110.0)*	**98.2** *(77.4-122.5)*
mean *(SD)*	**96.7** *(35.2)*	**96.8** *(53.8)*	**96.8** *(28.1)*	**99.3** *(33.3)*
MALES (n)	44	30	6	20
median *(Q1-Q3)*	**85.3** *(68.2-119.1)*	**82.7** *(50.0-96.6)*	**100.5** *(86.8-107.9)*	**97.3** *(73.4-123.0)*
mean *(SD)*	**94.8** *(41.2)*	**82.0** *(43.0)*	**98.0** *(33.5)*	**97.2** *(37.5)*
NICOTINE				
ALL SUBJECTS (n)	156	118	39	74
median *(Q1-Q3)*	**2.3** *(1.7-3.4)*	**2.3** *(1.7-3.6)*	**2.4** *(1.6-3.9)*	**2.3** *(1.8-3.6)*
mean *(SD)*	**3.3** *(3.0)*	**3.2** *(2.5)*	**3.1** *(2.3)*	**4.3** *(8.0)*
FEMALES (n)	112	88	33	54
median *(Q1-Q3)*	**2.4** *(1.7-3.4)*	**2.3** *(1.7-3.6)*	**2.4** *(1.8-3.5)*	**2.5** *(1.7-4.1)*
mean *(SD)*	**3.3** *(3.1)*	**3.2** *(2.5)*	**3.0** *(2.0)*	**5.0** *(9.2)*
MALES (n)	44	30	6	20
median *(Q1-Q3)*	**2.2** *(1.8-3.3)*	**2.4** *(1.9-3.6)*	**2.4** *(1.5-3.9)*	**2.3** *(2.1-2.5)*
mean *(SD)*	**3.1** *(2.8)*	**3.3** *(2.6)*	**3.7** *(3.7)*	**2.5** *(1.0)*

A = not exposed, B=exposed to others, not mother, C=exposed to mother, D=exposed to mother and others.