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PII: S0013-9351(20)31290-1

DOI: <https://doi.org/10.1016/j.envres.2020.110393>

Reference: YENRS 110393

To appear in: *Environmental Research*

Received Date: 25 June 2020

Revised Date: 22 October 2020

Accepted Date: 22 October 2020

Please cite this article as: Lidón-Moyano, C., Fu, M., Perez-Ortuño, R., Ballbè, M., Garcia, E., Martín-Sánchez, J.C., Pascual, J.A., Fernández, E., Martínez-Sánchez, J.M., Third-hand exposure at homes: assessment using salivary cotinine, *Environmental Research*, <https://doi.org/10.1016/j.envres.2020.110393>.

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CLM and EG analyzed the data and drafted the first manuscript. MF, MB, EF and JMMS contributed to the design and coordination of the study. All authors contributed substantially to the interpretation of the data and the successive versions of the manuscript. All authors contributed to the manuscript and approved its final version. JMMS conceived the study and is the principal investigator of the project.

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Third-hand exposure at homes: assessment using salivary cotinine

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Word count: 2,918

ABSTRACT

Background / Objectives: While exposure to secondhand smoke (SHS) is a well-established problem, exposure to third-hand smoke (THS) is scanty known and needs to be studied. The objective of this work is to characterize salivary cotinine concentrations among people who self-reported exposure to SHS and THS at home.

Methods: Cross-sectional study of a representative sample ($n = 736$) of the adult population (≥ 16 years) from the city of Barcelona carried out in 2013-2014. A questionnaire on tobacco use and passive exposure was administered, and a saliva sample was collected for cotinine determination. For this study, the information of the non-smoker participants who provided saliva sample ($n = 519$) was used. The geometric means (GM) and geometric standard deviations (GSD) of the cotinine concentration were compared according to the type of self-reported exposure at home: (1) Not exposed to SHS or THS; (2) Exposed to SHS and THS; and (3) Only exposed to THS. We used log-linear models to compare the cotinine concentration of each exposed group with respect to the unexposed group, adjusting for sex, age, educational level, and tobacco exposure in other settings.

Results: The GM of the salivary cotinine concentration was 0.34 ng/mL (GSD=0.16) among individuals reporting SHS and THS exposure, 0.22 ng/mL (GSD=0.15) among those reporting only THS exposure and 0.11 ng/mL (GSD=0.04) among those who declared not to be exposed to SHS nor THS (p -value for trend <0.001). The regression model showed a statistically significant increase in cotinine concentration among those exposed to SHS and THS (188.3% higher, 95% CI: 153.6%; 222.9%), and only exposed to THS (105.7% higher, IC95. %: 74.5%; 137.0%) when comparing with the unexposed group. No statistically significant differences in cotinine concentration were observed between those exposed to SHS and THS compared to the THS group (-25.8%, 95% CI: -69.5%; 17.9%).

Conclusions / Recommendations: People exposed to third-hand smoke at home had quantifiable cotinine levels in saliva. No differences in cotinine levels were found between those exposed to second-hand and third-hand smoke at home. The reduction of exposure to third-hand smoke at home should be put into the agenda of tobacco control.

Keywords: third-hand smoke, tobacco exposure, biomarker, smoke-free homes

INTRODUCTION

The term third-hand smoke (THS), also known as ‘residual tobacco smoke’ or ‘aged tobacco smoke’, refers to the combination of gases and particles persisting, in the indoor environment (including cushions and carpeting among other) and in smoker’s hair, skin and clothing, long after tobacco smoke has cleared from a room ^{1,2}. THS has been formally defined as residual tobacco smoke pollutants that remain on surfaces and in dust after tobacco has been smoked, are re-emitted back into the gas phase, or react with oxidants and other compounds in the environment to yield secondary pollutants ³.

THS constitutes a new public health concern as its components found in indoor dust and surfaces could be ingested, inhaled or even absorbed through the skin ⁴. Moreover, as it is a relatively new concept, research is still needed to define THS exposure health hazards, specially its long-term effects ⁵. In this regard, recent studies included in a systematic review showed an increase in risk of respiratory symptoms, in risk of cancer, and in mortality risk associated with living with a smoker due to THS ⁵.

Moreover, the scarce scientific evidence also reflects in the public awareness, and even healthcare professionals awareness, being still limited ⁵. On this subject, previous studies showed that only up to 1/3 of health professionals have heard about THS ⁶, and 43% of adult smokers ⁷, and 65% of non-smokers ⁷, agreed that THS harms children. The population lack of information regarding THS hinder non-smoker protection as smoke-free legislations include public and workplaces, but private places as home (and vehicles), where exposure to THS is high, are never or rarely included, falling upon individuals the decision to establish smoke-free home rules. This entails that individuals’ decision is based on protecting non-smokers from secondhand smoke (SHS) neglecting THS protection. Regarding Spain, in 2014, only 37.6% of households had complete smoke-free rules ⁸, which would protect non-smokers from SHS but they

might not completely avoid exposition to THS as tobacco particles persisting in smoker's hair, skin and clothing might be ingested, inhaled or absorbed by non-smokers when interacting with smokers and might also adhere to communal areas furniture such as cushions, couch or beds.

To the best of our knowledge, most of THS research focus on its components and concentrations in surfaces, but there is scarce literature using biomarkers to objectively quantify THS exposure in individuals. In this regard, cotinine, the main nicotine metabolite, has been widely used as a biomarker of tobacco exposure⁹. Cotinine concentration in biological fluids (blood, urine or oral fluid, widely referred to as saliva)¹⁰ indicate tobacco exposure over the previous 1-2 days¹¹. Therefore, the objective of this work is to characterize salivary cotinine concentrations among self-reported perception of exposure to SHS and THS, using salivary cotinine as a personal biomarker.

METHODS

We used the follow-up data of a cohort study from a representative sample of the adult population (≥ 16 years) of the city of Barcelona (Catalonia, Spain). The baseline study was carried out during the years 2004-2005¹² ($n = 1,245$) and follow-up took place in 2013-2014 ($n=736$)^{12,13}. From the baseline sample, we excluded 235 subjects, 150 after checking their data in the Insured Central Registry of Catalonia (101 died and 49 migrated out of the province of Barcelona) and another 85 without consent to be followed up or being minor (<18 years old) in 2004-2005 whose parents did not provide informed consent to be re-contacted. Follow-up was conducted between May 2013 and

February 2014. In total, 72.9% of the eligible sample agreed to participate, 18.5% refused to participate, 7.2% had moved elsewhere and 1.3% had died. The final sample included 736 individuals (Figure 1). The final sample was skewed as slightly older in comparison with the general population of Barcelona. For this reason, we weighted our data according to age distribution of the city of Barcelona to maintain its representativeness. Weights were calculated taking into account non-smokers only, therefore inferences weighted to the non-smokers population.

We obtained 9 ml of saliva sample (i.e. oral fluid) for cotinine analysis. Participants were asked to rinse their mouths and then suck a lemon candy (Smint) to stimulate saliva production. Saliva samples were frozen and sent to the 'Hospital del Mar' Medical Research Institute (IMIM) in Barcelona. Salivary samples were analyzed with liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) with multiple reaction monitoring¹⁴. The limit of quantification was 0.1 ng/mL and the limit of detection was 0.03 ng/mL (quantification error <15%). For cotinine concentrations below the limit of quantification (34.1% of the samples), a value of half the level of quantification (0.05 ng/mL) was assigned. For this study, we only used the non-smokers who provided saliva sample and information of the questionnaire (Figure 1) (n =519). We considered as non-smoker the person who declares to have never smoked or to have formerly smoked, and has a salivary cotinine concentration compatible with non-

smoking (≤ 10 ng/ml)¹⁵ (Figure 1). 177 (34.1%) out of the 519 samples were below the limit of quantification and were assigned 0.05 ng/mL.

Exposure to SHS at home was determined through two questions: “Currently, how many individuals usually smoke inside your home per day?” and “During the last week, how many cigarettes (per day) have been smoked in your presence inside your home?” Answers were gathered for typical working and non-working days. Based on these two questions and whether the subject lived with a smoker, we derived a variable of type of tobacco smoke exposure at home, to discriminate between THS and SHS exposure. Type of SHS exposure was classified as ‘SHS and THS exposed’ including those individuals who self-reported exposure to SHS at home, as in a real-world environment, individuals exposed to SHS are generally exposed to THS as well; ‘Only THS exposed’ including those individuals who lived with a smoker but did not report exposure to SHS at home; and ‘Not exposed’ including those individuals who did not live with a smoker nor self-reported exposure to SHS at home. The definition of type of tobacco smoke exposure at home did not take into account smoking rules at home. Moreover, we obtained information about the self-reported exposure to SHS at work, public transport and leisure time (binary variable of exposure to SHS in each setting). Exposure to SHS in other settings was defined as exposure in at least one of the above-mentioned settings. Finally we included information regarding smoke exposure at home including the relationship with the smoker (categorized as ‘partner’ or ‘other’: offspring, parents, siblings, and others); Smoking rules at home, classified as ‘complete’ when smoking was not allowed inside the house, ‘partial’ when smoking was allowed in some places inside the house, or ‘absent’ when smoking was allowed everywhere inside the house; Smoking places at home, classified as ‘outside and ventilated areas’ including balconies, courtyards, terraces, gardens, galleries, windows or other ventilated areas,

‘specific room’ including office, study, dressing room, smoker’s room, or other specific room, ‘communal areas’ including toilet, kitchen, dining room, living room or other communal areas; and tobacco smoke enters home from the outside, classified as ‘yes’ or ‘no’.

Given the skewed distribution of cotinine concentration, we calculated geometric means (GM) and their geometric standard error (GSE). We used log-linear models adjusted for sex, age, educational level and exposure to SHS in other settings to model the change percentage in salivary cotinine concentrations (after log 10 transformation), and their 95% confidence intervals. According to smoke exposure at home, we compared individuals exposed to SHS and THS, and individuals only exposed to THS, with non-exposed individuals, and we also compared individuals exposed to SHS and THS with individuals only exposed to THS. Moreover, the results were stratified by sex, age, educational level (categorized as low: unschooled, elementary school completed or uncompleted and special education; intermediate: high school and training cycles; and high: university education), exposure to SHS in other settings, for the first comparison, and smoke exposure at home variables for the second comparison. The statistical program used was R-3.0.2. The research and Ethics Committee of the Bellvitge University Hospital provided ethical approval for the study. This study meets the code of the Declaration of Helsinki.

RESULTS

The final sample of this study included 519 non-smokers, classified according to the type of tobacco smoke exposure at home as non-exposed (n=439; 84.6%), SHS and THS exposed (n=56; 10.8%), and only THS exposed (n=24; 4.6%). We observed a general increase, and gradient, in salivary cotinine concentration according to the type of exposure at home, being lowest among non-exposed at any type of tobacco smoke, and highest among SHS and THS exposed (Figure 2, panel A). In addition, differences in the distributions of cotinine concentration were observed among individuals only exposed to THS or exposed to SHS and THS at home when comparing with non-exposed individuals (Figure 2). However, cotinine concentration of SHS and THS exposed individuals were similarly distributed to individuals only exposed to THS (Figure 2).

Salivary cotinine concentrations are significantly higher among SHS and THS exposed at home, 188% (GM from 0.11 ng/ml to 0.34 ng/ml), and exposed only to THS at home, 106% (GM from 0.11 ng/ml to 0.22 ng/ml), when comparing with non-exposed at home (Table 1). These differences in cotinine concentration were also observed even after stratifying by sex, age, educational level, and SHS in other settings (Table 1).

There were no evidences supporting differences in salivary cotinine concentrations between SHS and THS exposed at home and only exposed to THS, neither in the raw nor in the adjusted model, being higher among individuals exposed to SHS and THS

(Table 2). Moreover, there was no evidence supporting these differences even after stratifying according to smoking rules at home, places allowed to smoke at home, and whether smoke from the outside reaches the home (Table 2). However, significant differences were found in salivary cotinine concentrations between SHS and THS exposed at home individuals and only exposed to THS individuals after stratifying according to the relationship with smoker, in the raw and in the adjusted model, specifically when the non-smoker and smoker were partners, being the raw estimated difference -62.8% (GM from 0.47 ng/ml among SHS and THS to 0.17 ng/ml among only THS exposed individuals). Moreover, among non-smokers who allowed smokers to smoke in specific rooms, saliva cotinine levels were 30.8% lower in THS only exposed persons compared to SHS and THS exposed persons in adjusted models (-30.8%, GM from 0.30 ng/ml among SHS to 0.39 ng/ml among THS exposed individuals).

Table 1. Geometric mean (GM) and geometric standard deviation (GSD), linear regression coefficient (% change) and adjusted linear regression coefficient (% change^a) and their 95% confidence interval (95% CIs) of salivary cotinine concentration (ng/mL) according to sociodemographic variables, and second-hand smoke exposure in settings

		Tobacco smoke exposure at home			% change ^a Only THS	% change ^a SHS and THS
	n	No (n=439)	Only THS (n=24)	SHS and THS (n=56)		
Overall	519	0.11 (0.04)	0.22 (0.15)	0.34 (0.16)	106 (53.7, 175)***	188 (109, 297)***
Sociodemographic characteristics						
Sex						
Men	226	0.11 (0.06)	0.26 (0.24)	0.35 (0.25)	128 (56.4; 232)***	190 (76.5; 377)***

other than home, stratified by type of tobacco smoke exposure at home.

Partial	51	0.26 (0.21) (n=31; 55.3%)	0.24 (0.18) (n=20; 83.3%)	-9.7 (-46.7; 52.9)	2.8 (-31.5; 54.0)
Absent	25	0.51 (0.23) (n=24; 42.9%)	- (n=1; 4.2%)		
Places allowed to smoke ^b					
Outside and ventilated areas	32	0.23 (0.22) (n=22; 71.0%)	0.20 (0.19) (n=15; 75.0%)	-13.3 (-50.7; 52.5)	5.6 (-36.8; 76.5)
Specific room	10	0.30 (0.21) (n=4; 12.9%)	0.39 (0.26) (n=6; 30.0%)	31.8 (-28.7; 143.7)	-30.8 (-34.5; -27.0)***
Communal areas	16	0.38 (0.42) (n=11; 35.5%)	0.29 (0.16) (n=5; 25.0%)	-23.9 (-67.6; 79.1)	-4.3 (-54.9; 102.7)
Smoke from the outside ^c					
Yes	57	0.36 (0.20) (n=15; 26.8%)	0.22 (0.18) (n=8; 33.3%)	-38.9 (-63.5; 2.5)	-33.8 (-65.9; 28.6)
No	23	0.29 (0.25) (n=41; 73.2%)	0.24 (0.28) (n=16; 66.7%)	-18.0 (-59.8; 67.1)	-36.4 (-69.2; 31.5)

^a: Adjusted for sex, age, educational level and exposure to SHS in any setting

^b: The percentages do not sum up 100% due to this information was asked through a multiple response question.

^c: Tobacco smoke enters home from the outside, classified as 'yes' or 'no'

*** p-value < 0.001, ** p-value < 0.01, * p-value < 0.05; Obtained by the log-linear model

Type of tobacco smoke exposure at home: classified as 'SHS exposed' including those individuals who self-reported exposure to SHS at home; 'THS exposed' including those individuals who lived with a smoker but didn't report exposure to SHS at home; and 'Not exposed' including those individuals who didn't live with a smoker nor self-reported exposure to SHS at home

DISCUSSION

Our study shows differences in salivary cotinine according to type tobacco smoke exposure at home, being salivary cotinine concentrations higher among SHS and THS, and only THS exposed individuals, when comparing with non-exposed ones. In addition, we found no difference in the salivary cotinine concentrations when comparing SHS and THS exposed individuals with only THS exposed individuals.

Our results are in line with a previous study conducted in England that compare the cotinine concentration in children who live in smoke-free homes, according to the smoking status of their parents, showing that cotinine concentration was high among children of smoking parents (non-smoking parent GM: 0.22 ng/ml; one smoking parent GM: 0.37 ng/ml; two smoking parents GM: 0.71 ng/ml) (Jarvis, M. J. et al., 2009). In the same line, another study showed elevated concentrations of nicotine on hands and cotinine in urine of nonsmokers residing in homes previously occupied by smokers¹⁷, and in nonsmoker individuals visiting a smoke-free casino up to 6 months after the implementation of the ban¹⁸. Our results support the idea that THS exposure in individuals is real, quantifiable and distinctly higher than non-exposed individuals.

According to our results, generally, no difference was found in the salivary cotinine concentrations of SHS and THS exposed individuals and only THS exposed individuals at home. However, compared with SHS and active smoking, existing evidence suggests that exposure to THS involves very different time profiles of exposure (i.e., low-level cumulative exposure over long periods vs. repeated exposures to high levels over short intervals), different pollutant concentrations in different media (i.e., surfaces and dust vs. primarily air), novel pollutants not found in SHS, and different relative contributions of exposure routes (i.e., inhalation vs. dermal vs. ingestion)². In fact, the persistence of THS in real-world residential settings has been demonstrated based on nicotine and 3-ethenylpyridine concentrations in air, dust, and surfaces in the days, weeks, and months

after the last smoking has taken place and some research support that the lifecycle of SHS compared with THS is brief (Bahl et al., 2014; Díez-Izquierdo, A. et al., 2018).

Consequently, health risks of THS may include some of those of SHS and active smoking as well as new ones not yet directly associated with tobacco smoke ².

In our study, regarding the salivary cotinine concentration comparison between SHS and THS exposed individuals, and only THS exposed individuals, evidence was found supporting differences in some stratified models. First, when the non-smoker and smoker were partners (either in the raw and in the adjusted model). Second, when non-smokers live in homes allowing smoking in specific rooms (in the adjusted model). In both cases the estimation of the difference suggested that SHS has greater effect than THS in terms of salivary cotinine concentration. These two scenarios are those which THS effect is expected to be higher (i.e. sharing bed with smoker, or smoking inside one specific room) and therefore, SHS might reflect the joint effect of direct exposure to tobacco smoke (SHS) and the indirect effect of THS. In addition, the results obtained in the 'specific room' category could be due to concentrated THS exposure in small and poorly ventilated rooms, that could be more easily transferred to non-smoker individuals. However, this result should be interpreted cautiously due to possible over-adjustment due to the small sample in this stratified model. Further studies should include this information to explore this hypothesis. Moreover, our results could be confounded by type of smoking rules at homes (complete vs partial), the places where it

is allowed to smoke, or the exposure to SHS in other setting, however, we are not able to explore this due to low sample size. Finally, there are other variables that could take a role in the comparison between SHS and THS, and only THS salivary cotinine, such as duration and intensity of tobacco smoke exposure, anyhow while this information is commonly included on SHS exposure studies, it is harder to define and measure duration and intensity of THS exposure. In this sense, future studies should work on the definition and measurement of THS exposure's duration and intensity.

The main limitation of our study is self-reported information to define levels of tobacco smoke exposure at home. In this regard, SHS and THS exposure are overly intertwined, hindering its differentiation. First, THS exposure cannot be completely separated from SHS, due to individuals exposed to THS might be also exposed to SHS still present in space (i.e. when smoke takes places in a room and individuals enter shortly after smoking ends). Second, individuals reporting not being exposed to SHS, might actually be unaware of their exposure (i.e. when smokers smoke outside the house without closing doors/windows, or when smokers light the cigarette inside the home). Actually, 20 (83.3%) of the 24 THS exposed individuals followed partial smoking rules. Nonetheless, among them, only 5 (25.0%) allowed smoking in communal areas, while 6 (30.0%) only allowed smoking in specific rooms and 15 (75.0%) only allowed smoking outside and in ventilated areas. Moreover, even though these individuals followed partial smoking rules, they declared not being exposed to SHS, which might indicate

that smoking is not taking place when the individuals are present. However, THS might be overestimated due to SHS non-reported exposure. Previous studies have shown that cotinine could be a specific biomarker of THS ²⁰. However, the discrimination of the concentration between SHS and THS could be difficult. For this reason, future studies should include the evaluation of environmental tobacco measures such as surface wipe sampling for nicotine to objectively define, and discriminate between, SHS and THS tobacco smoke exposure. In order to minimize this potential limitation, we used a face-to-face questionnaire with trained interviewers potentially increasing the internal validity of our results. Moreover, although smoking in many public settings is banned by the current smoking law ²¹, exposure to SHS and THS in other settings could still occur. We gathered self-reported information about the exposure in settings other than home and we compared the results with and without this information in the adjusted models and similar results were obtained; thus, we opted for not including them. In addition, smoking bans in Spain reduce SHS in other settings as they included indoor work, public transport, hospitality venues, and some outdoor areas, including hospital premises, educational campuses, and playgrounds. According to previous studies, significant difference was found between salivary cotinine when comparing self-declared SHS exposure (Yes vs No) at home, but no evidence was found neither at work, nor at public transport, nor at leisure time ²². Information about other non-cigarette tobacco product as SHS and THS sources was not available. However,

cigarette is the principal tobacco product used by smokers in comparison with other products such as pipes, hookah, or e-cigarettes. Actually, a national Spanish survey showed that 96.5% of smokers smoked cigarettes in 2017²³. Therefore, we do not expect that this limitation affects the internal validity of the study. Nevertheless, future research should include information regarding other non-cigarette tobacco products. Moreover, this study lacks other relevant variables such as race/ethnicity. However, our questionnaire includes a variable to identify the country of birth, although this does not imply race or ethnicity (99.0% of the participants were born in Spain). Another relevant limitation is the potential of participation bias due to the attrition of the cohort of participants; our data are, particularly, older than the population of the city of Barcelona. However, we weighted the sample to minimize this limitation and to generate estimations representative of the general population. We compared both unweighted and weighted results and they were similar. Other potential limitation is the cross-sectional nature of the data analyzed, which allow to establish associations but not to infer causality.

In conclusion, our results support the idea that THS is a source of tobacco exposure for non-smokers living with a smoker, even in households with voluntary smoke-free rules.

The reduction of exposure to THS has to be put on the agenda of tobacco control.

Research is needed to explore solutions on how to reduce THS exposure such as the removal of nicotine from hands of smokers or those exposed to SHS/THS, and outside

smoking practices to reduce the amount of THS that enters homes and buildings.

Moreover, information campaigns will be needed to raise awareness regarding THS exposure among individuals and healthcare professional.

DECLARATION OF INTERESTS

Authors declare that they have no conflicts of interest.

FUNDING

This project was partially funded by the Instituto de Salud Carlos III, Subdirección General de Evaluación, Government of Spain (PI12/01114 and PI12/01119), co-funded by ISCIII-Subdirección General de Evaluación and by FEDER funds/ European Regional Development Fund (ERDF) –a way to build Europe-. The Group of Evaluation of Health Determinants and Health Policies (CL, EG, JCMS, JMMS) receives support from the Ministry of Universities and Research, Government of Catalonia (grant 2017SGR609) from the Government of Catalonia. The Tobacco Control Research Group (MF, MB, JMMS, EF) receives support from the Ministry of Universities and Research, Government of Catalonia (grant 2017SGR319) from the Government of Catalonia. EF is partly supported by the Instituto de Salud Carlos III, Government of Spain, co-funded by the European Regional Development Fund (FEDER; INT16/00211 and INT17/00103).

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Figure1. Flow chart of sample followed-up from Barcelona, Spain, in 2013-2014.

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Figure 2. Distribution of salivary cotinine in non-smokers (ng/ml, in log scale) according to their individual exposure at home ('SHS and THS exposed': those individuals who self-reported exposure to SHS at home; 'Only THS exposed': those individuals who lived with a smoker but did not report exposure to SHS at home; 'Not exposed': those individuals who did not live with a smoker nor self-reported exposure to SHS at home).

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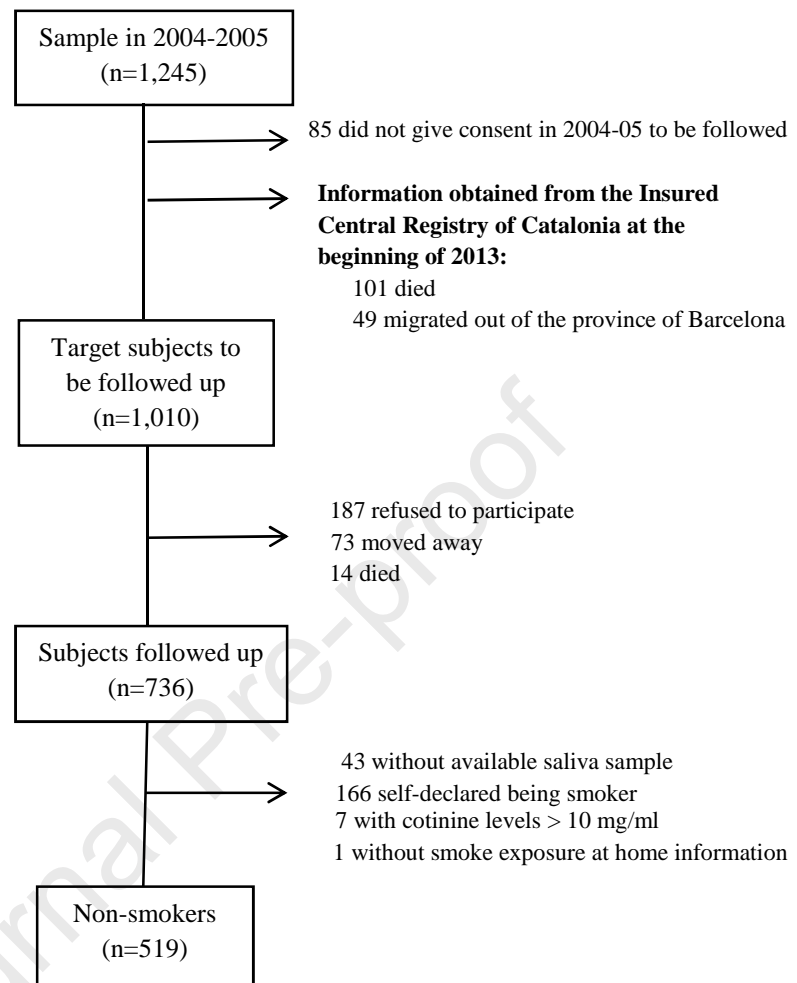
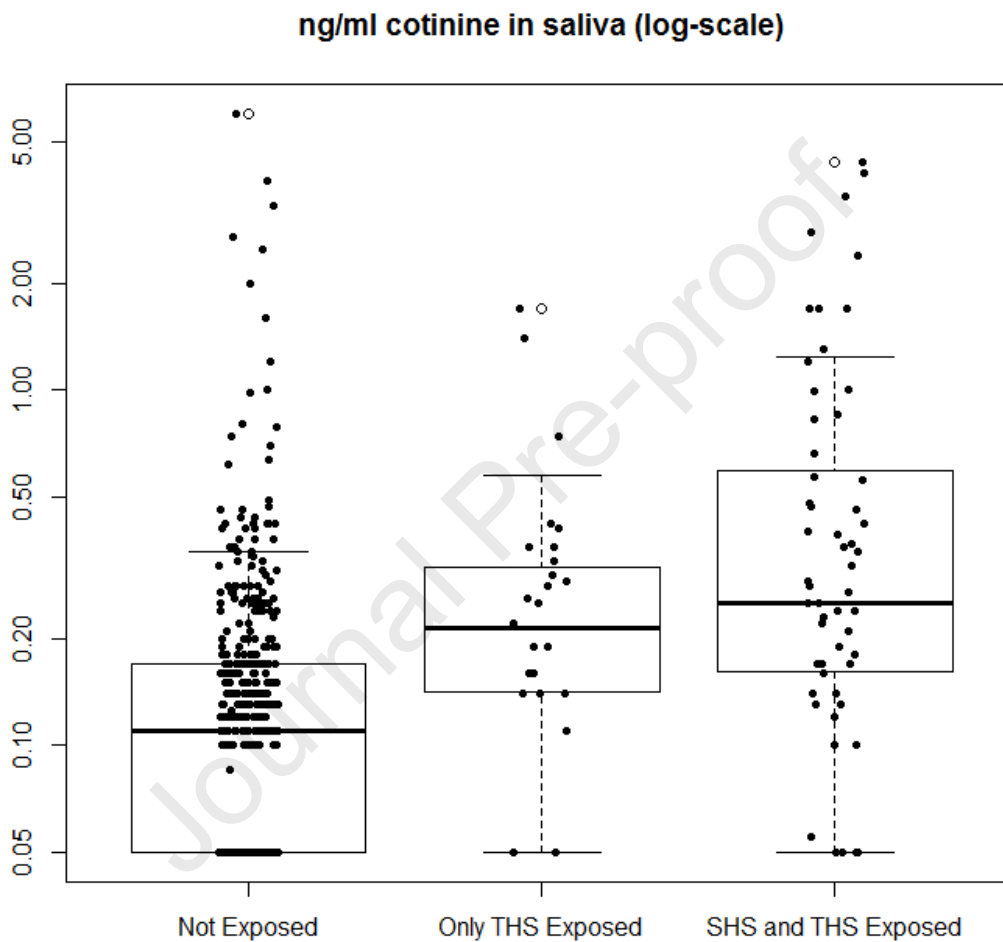
Figure1. Flow chart of sample followed-up from Barcelona, Spain, in 2013-2014.

Figure 2. Boxplot (and individual data) of cotinine in saliva (ng/ml) according to smoke exposure at home, classified as ‘SHS and THS exposed’ including those individuals who self-reported exposure to SHS at home; ‘Only THS exposed’ including those individuals who lived with a smoker but didn’t report exposure to SHS at home; and ‘No exposed’ including those individuals who didn’t live with a smoker nor self-reported exposure to SHS at home.



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- THS refers to the residuals lingering after tobacco smoke has cleared from a room.
- Scarce literature uses biomarkers to quantify THS exposure in individuals.
- Our study shows salivary cotinine differences between tobacco smoke exposures.
- Salivary cotinine was higher among SHS, and THS exposed individuals.
- No differences were found between SHS and THS exposed individuals.

DECLARATION OF INTERESTS

Authors declare that they have no conflicts of interest.

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