

Brief original article

## Secondhand exposure to aerosol from electronic cigarettes: pilot study of assessment of tobacco-specific nitrosamine (NNAL) in urine



Jose M. Martínez-Sánchez<sup>a,b,c,\*</sup>, Montse Ballbé<sup>b,c,d,e,f</sup>, Raúl Pérez-Ortuño<sup>g</sup>, Marcela Fu<sup>b,c,f</sup>, Xisca Sureda<sup>b,c,g</sup>, José A. Pascual<sup>h,i</sup>, Armando Peruga<sup>j</sup>, Esteve Fernández<sup>b,c,d,f</sup>

<sup>a</sup> Group of Evaluation of Health Determinants and Health Policies, Universitat Internacional de Catalunya, Sant Cugat del Vallès (Barcelona), Spain

<sup>b</sup> Tobacco Control Unit, Cancer Prevention and Control Program, Institut Català d'Oncologia, L'Hospitalet de Llobregat (Barcelona), Spain

<sup>c</sup> Cancer Prevention and Control Group, Institut d'Investigació Biomèdica de Bellvitge - IDIBELL, L'Hospitalet de Llobregat (Barcelona), Spain

<sup>d</sup> Catalan Network of Smoke-free Hospitals, L'Hospitalet de Llobregat (Barcelona), Spain

<sup>e</sup> Addictions Unit, Institute of Neurosciences, Hospital Clínic de Barcelona, Barcelona, Spain

<sup>f</sup> Department of Clinical Sciences, Universitat de Barcelona, Barcelona, Spain

<sup>g</sup> Social and Cardiovascular Epidemiology Research Group, School of Medicine, University of Alcalá, Alcalá de Henares (Madrid), Spain

<sup>h</sup> Hospital del Mar Medical Research Institute - IMIM, Barcelona, Spain

<sup>i</sup> Department of Experimental and Life Sciences, Universitat Pompeu Fabra, Barcelona, Spain

<sup>j</sup> Tobacco Free Initiatives, World Health Organization, Geneva, Switzerland

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### ABSTRACT

**Objective:** To assess the levels of a tobacco-specific nitrosamine (NNAL) in non-smokers passively exposed to the second-hand aerosol (SHA) emitted from users of electronic cigarettes (e-cigarettes).

**Method:** We conducted an observational study involving 55 non-smoking volunteers divided into three groups: 25 living at home with conventional smokers, 6 living with e-cigarette users, and 24 in control homes (smoke-free homes). We obtained urine samples from all volunteers to determine NNAL.

**Results:** We detected NNAL in the urine of volunteers exposed to e-cigarettes (median: 0.55 pg/mL; interquartile range: 0.26–2.94 pg/mL). The percentage of urine samples with quantifiable NNAL differed significantly among the three groups of homes: 29.2%, 66.7% and 76.0%, respectively ( $p = 0.004$ ).

**Conclusions:** We found NNAL nitrosamine in urine samples from people exposed to SHA from e-cigarettes. However, these results could be confirmed with more studies with larger sample sizes.

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## Exposición pasiva al aerosol del cigarrillo electrónico: estudio piloto de evaluación de la nitrosamina específica del tabaco (NNAL) en orina

### RESUMEN

**Objetivo:** Evaluar los niveles de nitrosamina específica del tabaco (NNAL) en no fumadores expuestos pasivamente al aerosol emitido por usuarios de cigarrillo electrónico.

**Método:** Estudio observacional de una muestra de 55 voluntarios no fumadores divididos en tres grupos: 25 que vivían en una casa con un fumador de tabaco convencional, 6 que vivían en una casa con un usuario de cigarrillo electrónico y 24 que vivían en casas controles (hogares libres de humo). Se obtuvo una muestra de orina de todos los voluntarios para determinar las concentraciones de NNAL.

**Resultados:** Se detectaron valores de NNAL en los voluntarios expuestos al cigarrillo electrónico (mediana: 0,55 pg/ml; rango intercuartílico: 0,26–2,94 pg/ml). El porcentaje de voluntarios con concentraciones cuantificables de NNAL fue estadísticamente diferente entre los tres grupos de casas: 29,2%, 66,7% y 76%, respectivamente ( $p = 0,004$ ).

**Conclusiones:** Se encontraron valores de NNAL en los no fumadores expuestos pasivamente al aerosol del cigarrillo electrónico. Estos resultados tienen que confirmarse con muestras más grandes.

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#### Palabras clave:

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### Introduction

As electronic cigarettes (e-cigarette) use has been grown, more concerns have appeared about the exposure of bystanders to secondhand aerosol (SHA) from these devices. This exposure results

\* Corresponding author.

E-mail address: [jmmartinez@uic.es](mailto:jmmartinez@uic.es) (J.M. Martínez-Sánchez).

**Table 1**  
Characteristics of the e-liquids and e-cigarettes in this study.

E-liquid characteristics				E-cigarettes characteristics	
Brand	Flavour	Nicotine concentration (mg) <sup>a</sup>	Nicotine consumption per day <sup>b</sup>	Type	Brand
Totally Wicked	Menthol	11	62.7 mg	Cigalike	KangerTech KR808D-1
Totally Wicked	Marlboro	24	48 mg	e-Go	E-go C Totally Wicked
Puff	Menthol	18	180 mg	e-Go	Puff
Puff	Keen Tobacco	9	27 mg	e-Go	e-Go-T Upgrade Joyetech
Free Life	Mint	6	19.2 mg	e-Go	e-Go-T Joyetech
Unknown	Mint	6	12.85 mg	e-Go	Unknown

<sup>a</sup> As stated by the e-liquid manufacturer.

<sup>b</sup> As reported by the e-cigarettes users.

when the aerosol inhaled by users (firsthand aerosol) is exhaled into the air where it may be breathed by non-users.<sup>1</sup> This exposure is worrying because several studies have found toxic and carcinogenic substances in the aerosol (both firsthand and secondhand) generated by e-cigarettes.<sup>1,2</sup>

Tobacco-specific nitrosamines have been found in the aerosol generated by e-cigarettes<sup>3,4</sup> and also in some brands of e-cigarette liquids,<sup>5,6</sup> although not all of the studies have detected tobacco-specific nitrosamines in the samples studied,<sup>7</sup> a finding that indicates that important differences between brands may exist. Despite the potential presence of toxic substances in these aerosols, exposure to SHA from e-cigarettes has received scant attention according to a recent systematic review.<sup>2</sup>

For these reasons, we conducted the present study to assess the levels of NNAL, nitrosamine associate with lung cancer,<sup>8</sup> in urine samples in a group of non-smokers exposed to SHA from e-cigarette users in their homes under real-life conditions. Moreover, we compared these concentrations with those of non-smokers passive exposed to conventional cigarettes in their homes and with those of non-smokers not exposed to aerosol from e-cigarettes neither conventional cigarettes in their homes.

## Method

We conducted a study of passive exposure to electronic and conventional cigarettes in real-use conditions. A tobacco-specific nitrosamine (NNAL) was determined in urine samples from a group of volunteers. We recruited a convenience sample of 55 non-smoking volunteers from different homes, distributed as follows: 25 living at home with conventional smokers, 6 living with e-cigarette users, and 24 from control homes (without the presence of either conventional smokers or e-cigarette users). We also enrolled the 6 e-cigarette users who lived with the non-smoking volunteers. Participants living with e-cigarette users or smokers, and the e-cigarette users, provided self-reported data affirming that their only source of exposure to SHA from e-cigarette or tobacco smoke during the one-week study period was in their home and also confirmed that they did not use any tobacco products. These were the conditions for study inclusion that volunteers (non-smokers and e-cigarette users) agreed to at study enrolment. The self-reported lack of exposure in settings other than their homes (work, leisure time, and transport) was confirmed by a personal interview. In addition, all volunteers, including the 6 e-cigarettes users, declared that they did not use other tobacco products or nicotine replacement therapy during the study period. This pilot study is part of a large project which the main objective is to assess the impact of Spanish legislations and the passive exposure to secondhand smoke of conventional cigarettes at home. We conducted this pilot study due to the increasing popularity of the e-cigarettes and the lack of evidence worldwide about passive exposure to SHA from e-cigarettes. The fieldwork was conducted in Barcelona, Spain, in 2012.

The characteristics of the e-liquids and e-cigarettes used in this study are described in [Table 1](#).

We obtained urine samples for NNAL analysis by liquid chromatography coupled with tandem mass spectrometry with multiple reaction monitoring (LC/MS/MS). We measured the NNAL concentration in picograms per milligram of creatinine (pg/mg) because the NNAL concentrations were adjusted for creatinine. The limit of quantification (LOQ) of NNAL was 0.25 pg/mg in 5 mL of urine for a 1 mg/mL of creatinine excretion.

## Data analysis

We calculated the percentage of the volunteers with NNAL concentrations over the LOQ. For the samples with NNAL concentrations below the LOQ, we assumed the half of the LOQ value to compute the medians and interquartile ranges (IQRs). Given the skewed distribution of NNAL we compared the concentration among three types of homes with the Kruskal Wallis test. Then, we compared the concentration of NNAL among groups by means of the Chi square test and the Wilcoxon test for independent samples. We also calculated the Spearman correlation among NNAL concentrations of the non-smokers exposed to e-cigarettes and users of e-cigarettes who lived with them.

## Results

Statistically significant differences were found among the three groups of homes in the percentage of volunteers with quantifiable levels of NNAL in urine: 29.2%, 66.7%, and 76% ([Table 2](#)). After adjusting for multiple comparisons, differences in NNAL urine concentration were on the borderline of statistical significance between non-smokers in control homes and those exposed to tobacco smoke at home ([Table 2](#)).

As shown in [Table 3](#), we quantified traces of NNAL in the urine samples of four out of the six volunteers exposed to SHA from e-cigarette users. Spearman's correlation of the NNAL levels in urine of the e-cigarettes users and the volunteers exposed to SHA from e-cigarette users was 0.943 ( $p = 0.005$ ).

From six e-cigarette users, NNAL was quantifiable in the urine sample (median: 2.86 pg/mg; IQR: 0.36-6.92 pg/mL) (data not shown).

## Discussion

Our results show that there are quantifiable levels of NNAL in urine among bystanders exposed to SHA from e-cigarette users at home. Moreover, we also found a strong correlation between NNAL levels in urine among e-cigarette users and those of the non-smokers exposed to SHA at home from e-cigarette users. A previous study conducted in 698 individuals (532 of them non-smokers)

**Table 2**

Percentages of samples with detectable levels of NNAL and median (pg/mL) and interquartile range of NNAL in urine samples of the non-smokers volunteers according to passive exposure at home (non-exposed or control homes, exposed to secondhand aerosol from e-cigarettes users, and exposed to tobacco smoke).

	n	NNAL in urine samples <sup>a</sup>	
		Percentage of samples with detectable NNAL	Median (IQR)
Non exposed (control homes)	24	29.2%	0.33 (0.16 - 0.51)
Exposed to e-cigarettes' aerosol	6	66.7%	0.55 (0.26 - 2.94)
Exposed to conventional cigarettes	25	76.0%	0.46 (0.29 - 1.11)
p-value <sup>b</sup>		0.004	-
p-value <sup>c</sup>		-	0.040
p-value <sup>d</sup>		-	0.129
p-value <sup>e</sup>		-	0.017
p-value <sup>f</sup>		-	0.865

IQR: interquartile range.

<sup>a</sup> Adjusted for urinary creatinine.

<sup>b</sup> Pearson's Chi square.

<sup>c</sup> Comparison among volunteers from the three types of homes (non-exposed or smoke-free homes, exposed to e-cigarettes' aerosol, and conventional cigarettes) by Kruskal Wallis test.

<sup>d</sup> Comparison between volunteers from smoke-free homes (control homes) and e-cigarettes homes by Wilcoxon test for independent samples.

<sup>e</sup> Comparison between volunteers from smoke-free homes (control homes) and conventional cigarettes homes by Wilcoxon test for independent samples.

<sup>f</sup> Comparison between volunteers from e-cigarettes homes and conventional cigarettes homes by Wilcoxon test for independent samples.

**Table 3**

Levels of NNAL in the samples of urine of the e-cigarettes user and non-users exposed to secondhand aerosol from e-cigarettes users.

	NNAL	
	E-cigarette users	Exposed to secondhand aerosol from e-cigarette users
Urine samples (pg/mL) <sup>a</sup>		
Home 1	0.37	<0.56
Home 2	0.33	<0.44
Home 3	6.1	3.0
Home 4	0.42	0.44
Home 5	5.3	0.67
Home 6	9.4	2.9

<sup>a</sup> Adjusted for urinary creatinine.

The limits of quantification were 0.25 pg/mL for NNAL in 5 mL of urine for a 1 mg/mL of creatinine excretion.

also found levels of NNAL in saliva among non-smokers exposed to secondhand smoke.<sup>9</sup>

Vogel et al.<sup>10</sup> found that the levels of NNAL in urine (without adjusting for creatinine) ranged from 6.27 to 12.54 pg/mL, among non-smokers exposed to secondhand smoke from conventional cigarettes. In our study, the levels were lower in participants who were passively exposed to e-cigarettes (median: 0.47 pg/mL; range: 0.125–3.2 pg/mL) and to conventional cigarettes (median: 0.52 pg/mL; range: 0.125–8.3 pg/mL). These differences could be explained partially by the differences on the sample size of both studies and the inclusion criteria of our study (at home was the only source of exposure of our non-smokers volunteers and e-cigarette users in the last week). Moreover, other potential explanation could be the difference in the duration and intensity of exposure among studies, and the measurement bias inter-laboratory. Although the levels of NNAL among non-smokers exposed passively to e-cigarettes are very low<sup>8</sup> there is no safety level of exposure and the risk increases with the intensity and duration of exposure.<sup>11,12</sup>

The average NNAL concentration in urine (unadjusted for creatinine) of smokers is around 300 pg/mL.<sup>13</sup> We also found levels of NNAL in users of electronic cigarettes (median without adjusting for creatinine: 2.6 pg/mL; range: 0.33–9.7 pg/mL). Compared to smokers, these results are orders of magnitude lower<sup>10,13</sup> but this magnitude was similar to those found in e-cigarette users in a previous study.<sup>14</sup>

Our results should be interpreted with caution due to the small number of volunteers exposed to e-cigarette users at home and the differences brands of e-cigarettes. In addition, although one

inclusion criterion was that participants' only permitted exposure to smoking during the study period was at home, we cannot be sure that participants' did not receive any additional unreported exposure. In an effort to avoid this limitation all participants did not use nicotine replacement therapy and they were instructed to avoid exposure to e-cigarettes and conventional cigarettes except at home.

In conclusion, we found quantifiable levels of NNAL in urine samples of non-smokers passively exposed to SHA from e-cigarette users. However, these results could be confirmed with more studies.

### What is known about the topic?

Interest, popularity and awareness of electronic cigarettes (e-cigarettes) have substantially increased in recent years. As e-cigarette use has been grown, more concerns have appeared about the exposure of bystanders to secondhand aerosol from these devices. This exposure results when the aerosol inhaled by users (firsthand aerosol) is exhaled into the air where it may be breathed by non-users.

### What does this study add to the literature?

Our results show that there are quantifiable levels of NNAL in urine among bystanders exposed to secondhand aerosol from e-cigarette users in the home. We also found a very strong correlation between carcinogenic NNAL levels in urine among e-cigarette users and those of the non-smokers exposed to secondhand aerosol at home from e-cigarette users.

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### Transparency declaration

The corresponding author on behalf of the other authors guarantee the accuracy, transparency and honesty of the data and information contained in the study, that no relevant information has been omitted and that all discrepancies between authors have been adequately resolved and described.

## Authorship contributions

M. Ballbè and J.M. Martínez-Sánchez conceived the study, conducted the fieldwork, prepared the database, analysed the data and drafted the manuscript. M. Ballbè, J.M. Martínez-Sánchez, X. Sureda, M. Fu, R. Pérez-Ortuño, J.A. Pascual, A. Peruga and E. Fernández contributed substantially to the conception, design, and interpretation of data. All authors contributed to the manuscript and approved its final version.

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## Conflicts of interest

None.

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