1 **Exposure to Nitrosamines in Thirdhand Tobacco Smoke** **Cancer Risk in Non-**

2 **increases Smokers**

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19   **Abstract**

20   In addition to passive inhalation, non-smokers, and especially children, are exposed to residual
21   tobacco smoke gases and particles that are deposited to surfaces and dust, known as thirdhand
22   smoke (THS). However, until now the potential cancer risks of this pathway of exposure have
23   been highly uncertain and not considered in public health policy. In this study, we estimate for the
24   first time the potential cancer risk by age group through non-dietary ingestion and dermal
25   exposure to carcinogen N-nitrosamines and tobacco-specific nitrosamines (TSNAs) measured in
26   house dust samples. Using a highly sensitive and selective analytical approach we have
27   determined the presence of nicotine, eight N-nitrosamines and five tobacco-specific nitrosamines
28   in forty-six settled dust samples from homes occupied by both smokers and non-smokers. Using
29   observations of house dust composition, we have estimated the cancer risk by applying the most
30   recent official toxicological information. Calculated cancer risks through exposure to the observed
31   levels of TSNAs at an early life stage (1 to 6 years old) exceeded the upper-bound risk
32   recommended by the USEPA in 77 % of smokers and 64 % of non-smokers homes. The
33   maximum risk from exposure to all nitrosamines measured in a smoker occupied home was one
34   excess cancer cases per one thousand population exposed.

35   The results presented here highlight the potentially severe long-term consequences of THS
36   exposure, particularly to children, and give strong evidence of its potential health risk and,
37   therefore, they should be considered when developing future environmental and health policies.

38   **Keywords:** thirdhand tobacco smoke; cancer risk assessment; N-nitrosamines; tobacco-specific
39   nitrosamines (TSNAs)
40

1. Introduction

Each year 600,000 people die worldwide from exposure to environmental tobacco smoke (Oberg et al. 2011), also called second hand smoke (SHS). As numerous countries have introduced smoking bans in public places (WHO 2010), domestic environments have become the main sources of passive smoking exposure (World Health 2007). However, the risks of tobacco exposure do not end when a cigarette is extinguished and non-smokers, especially children, are also at risk through contact with surfaces and dust contaminated with residual smoke gases and particles, the so-called third hand smoke (THS) (Matt et al. 2004; Matt et al. 2011a). Over 40% of children have at least one smoking parent (Oberg et al. 2011) and numerous studies have demonstrated the association between prenatal and early stage childhood diseases and the smoking habits of their parents (Cook and Strachan 1999). Although there is a general public awareness about the harms of SHS, the general public are more sceptical about THS, with a study in 2009 finding that 62.5 % of non-smokers and 43 % of smokers agreed that THS harms children (Winickoff et al. 2009). A study of parents' attitudes found that fathers and heavy smokers (>10 cigarettes per day) were less likely to believe that THS was harmful (Drehmer et al. 2012). The specific role of THS in tobacco-related illnesses has been questioned by the public health community (Matt et al. 2011a), however, a recent study demonstrated that chemical species associated with THS are genotoxic in human cell lines (Hang et al. 2013). Evidence of the chemical toxicity of THS is necessary to improve understanding of the risks of THS-polluted environments and to design educational strategies for families and the general public to allow them to make more informed decisions.

Nicotine is the most abundant organic compound emitted during smoking (Sleiman et al. 2010) and is considered a good marker of tobacco exposure. After cigarette smoking, nicotine deposits almost entirely on indoor surfaces, where it can be released again to the gas phase or react with ozone, nitrous acid and other atmospheric oxidants producing secondary pollutants, such as tobacco-specific nitrosamines (TSNAs) (Sleiman et al. 2010). Figure 1 shows the structures and reaction pathways of formation of the main TSNAs. Of the TSNAs identified, N′-nitrosonornicotine (NNN) and 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK) are the most prevalent and most active carcinogens in tobacco products (Hecht and Hoffmann 1988; Hecht 2003), inducing tumours in lung, liver, nasal cavities, oesophagus and exocrine pancreas, and are classified as carcinogenic for humans (Group 1 International Agency for Research on Cancer, IARC) ((IARC) 2007). Whilst some TSNAs can be directly produced during tobacco smoking, several studies have suggested that airborne NNK concentrations in sidestream cigarette smoke can increase by 50-200% per hour during the first 6h after cigarettes are extinguished (Schick and Glantz 2007). Moreover, NNK can further degrade and its main metabolite, 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanol (NNAL), is considered to have similar adverse health effects (Hecht 2008).

Given the low volatility of TSNAs and the high levels of nicotine typically found in environments contaminated with tobacco, TSNAs can persist for weeks to months in THS. Several studies have

79 detected nicotine in indoor dust and surfaces (Kim et al. 2008; Matt et al. 2011a) and recent
80 studies have demonstrated a correlation between the number of cigarettes smoked and the
81 presence of nicotine and polycyclic aromatic hydrocarbons (PAHs) (Hoh et al. 2012) in settled
82 house dust. The health risk from THS will be substantially controlled however by the prevailing
83 levels of TSNAs. Whilst these species have been seen directly in tobacco smoke (Mahanama and
84 Daisey 1996), there has been no measurement of their presence in THS.

85 Here we report the detailed determination of nicotine and five TSNAs (indicative of a tobacco
86 smoking source) and eight non-specific volatile N-nitrosamines (commonly released during
87 tobacco smoking, but likely to have additional environmental sources), in settled house dust
88 samples from homes occupied by smoking or non-smoking occupants. The complete list of these
89 target compounds is shown in Table 1. We have calculated the cancer risk related to exposure to
90 observed concentrations of the carcinogen N-nitrosamines and TSNAs through non-dietary
91 ingestion and dermal exposure by age group. For the first time, we use ambient observations to
92 constrain risk assessment estimations of exposure to these carcinogens in THS, based on real-
93 world measurements.

2. Material and methods

2.1. Sample collection and preparation

A total of 46 house dust samples were collected from private homes, using conventional vacuum cleaners in regular use in households between October 2011 and May 2012 in the area of Tarragona (north-eastern Spain). We have selected those samples whose residents have lived in their current home for at least one year. A questionnaire was designed to collect information about the house and any activity that might affect chemical loading (see Supplementary Material, Table S1). A summary of the collected information can be found in Table 2. As seen in the Table, most of the samples were flats in urban areas with low to moderate traffic intensity (up to 14,041 vehicles per day, Spanish Ministry of Public Works, personal communication). Around half (48%) of the samples were characterized as from smokers' homes, where at least one occupant was a tobacco smoker, including those whose occupants do not smoke inside the home. The mean number of cigarettes smoked per day in this group was 17 including cigarettes smoked both inside the home and at other locations outside the homes. The remainder of the samples (52%) were classified as non-smokers' homes, according to the survey information. See Table 2 for other relevant characteristics relevant of the homes included in this study.

The collected dust was sieved with an acetone washed stainless steel sieve and the fraction under 100 µm was stored in glass vials, preserved from light and kept at 4°C until analysis.

2.2. Sample extraction and chromatographic analysis

We have extracted 500 mg of the sieved dust samples by pressurised liquid extraction (PLE) using ASE 200 equipment (Dionex, Sunnyvale, CA, USA) with ethyl acetate as extraction solvent and silica as clean-up sorbent. Extracts were preserved from light and frozen at -20°C until analysis. Under the optimized extraction conditions, recoveries for most compounds were higher than 80%. Complete information about the PLE extraction conditions, their optimization and validation can be found in a previous study (Ramírez et al. 2012).

House dust is a complex matrix containing hundreds of inorganic and organic compounds. To improve selectivity and sensitivity we have analysed the extracts by comprehensive gas chromatography coupled with a nitrogen chemiluminiscence detector (GC×GC-NCD) that consists of a 7890 gas chromatograph, a 255 Nitrogen Chemiluminiscence Detector, both from Agilent (Palo Alto, CA, USA) and a quad-jet dual stage modulator from LECO (St. Joseph, MI, USA). The first column was a non-polar BPX5 (30 m × 0.25 mm × 0.25 µm, 5% diphenyl, 95% dimethylpolysiloxane) and the second column a BPX50 (1.5 m × 0.10 mm × 0.10 µm, 50% diphenyl 50% dimethylpolysiloxane) both from SGE Analytical Science (VIC, Australia). Analysis were performed by injecting 1 µL of the dust extracts, at 200°C in splitless mode, at a helium constant flow of 1 mL min^{-1}. First dimension oven temperature program started at 40°C, hold for 2

129 min, 5°C min^{-1} to 100°C, hold 4 min and 5°C min^{-1} to 300°C for 2 min. The modulator and second

130 oven temperature were 15°C above the first dimension oven and the modulation period was 5 s.

131 *2.3. Quality assurance*

132 Settled dust samples were extracted within one week after collection. Blanks of every step of the

133 analytical process were analysed for every five extracted samples and no detectable amounts of

134 the target compounds were found in the blanks. A subset of the samples (20%) were extracted

135 and analysed in triplicate, with an observed precision less than 8% RSD. Limits of detection

136 (LOD) ranged between 2.5 and 16 ng g^{-1}. More information about quality assurance and figures of

137 merits of the analytical method can be found in a recent publication (Ramírez et al. 2012).

138 *2.4. Cancer risk assessment*

139 Human exposure to THS is through non-dietary ingestion of settled house dust, dermal absorption

140 from the dust attached to fabrics and surfaces, and the possible inhalation of THS chemicals

141 revolatilised into the gas phase or those partitioned to breathable particles (Matt et al. 2011a). In

142 this study we have analysed the dust fraction under 100 μm diameter and, therefore, we have

143 considered ingestion and dermal absorption as the main pathways of human exposure to this

144 THS contaminated dust. The potential risk associated with this type of exposure is dependant on

145 age. Children, especially toddlers, are most at risk from non-dietary ingestion due to a number of

146 factors including: they spend relatively more time indoors; they engage in activities close to the

147 floor; they have hand-to-mouth behaviours; and they are more vulnerable to chemical exposure

148 because of their immature metabolism (USEPA 2008).

149 Table 1 shows the toxicological data relevant for this study including IARC classifications of the

150 target tobacco-related compounds (IARC 2013) and the oral slope factor values of the

151 carcinogenic ones. Cancer risk was estimated for the ten carcinogenic target nitrosamines, whose

152 toxicological values have been established by an official agency (NDMA, NMEA, NDEA, NDPA,

153 NPyr, NMor, NPip, NDBA, NNN and NNK) (IRIS 2013; OEHHA 2007). Oral slope factor values

154 were extracted from databases provided by the Integration Risk Information System (IRIS) (IRIS

155 2013) from the United States Environmental Protection Agency (USEPA) and the California Office

156 of Environmental Health Hazard Assessment (OEHHA) (OEHHA 2007), giving priority to IRIS

157 values.

158 We calculated the cancer risk by non-dietary ingestion using the following equation (USEPA 2004,

159 2005)

$$Risk_{ingestion} = \sum_{i=1}^{n} \frac{C_i \cdot IR \cdot CF \cdot EF \cdot ED}{BW \cdot AT} \cdot SF_i \cdot ADAF \qquad [1]$$

160 where C_i is the concentration (mg kg^{-1}) in the settled house dust samples of each of the 10

161 carcinogen nitrosamines considered in this study (*i*); *IR* is the Ingestion Rate (mg day^{-1}) by age

162 group; *CF* is the Correction Factor (10^{-6} kg mg^{-1}); *EF* is the Exposure Frequency (days year^{-1}); *ED*

163 is the Exposure Duration (years); *BW* is the average Body Weight; *AT* is the Average Time of life
164 (25550 days, corresponding to 70 lifetime years); SF_i is the oral Slope Factor [(mg×kg×day)$^{-1}$]
165 specific for each carcinogen; and *ADAF* is the default Age-Dependant Adjustment Factor
166 (unitless) that correct the non-age-specific slope factors. The values for these parameters (age
167 intervals: birth to <1; 1 to <6; 6 to <21; and 21 to 70) were selected according to the USEPA
168 criteria for dust exposure (USEPA 2011), except for the body weight for adults that is from the
169 National Institute of Statistics of the Spanish government (INE 2001). These values are shown in
170 the Supplementary Material, Table S2.

171 Cancer risk from dermal exposure was calculated using Equation 2 (USEPA 2004, 2005):

$$Risk_{dermal} = \sum_{i=1}^{n} \frac{C_i \cdot CF \cdot AF \cdot ABS \cdot EV \cdot SA \cdot EF \cdot ED}{BW \cdot AT} \cdot \frac{SF_i}{ABS_{GI}} \cdot ADAF \qquad [2]$$

172 Where *AF* is the Adherence Factor (mg cm^{-2} per event) by age interval; ABS is the Absorption
173 Fraction (unitless); EF is the Event Frequency (event day^{-1}); SA is the body surface area (cm^{-2});
174 and ABS_{GI} is the fraction of carcinogen Absorbed in Gastrointestinal tract (unitless), that has been
175 considered as 1 for all age groups (USEPA 2004). The values of these parameters were extracted
176 from the USEPA Risk Assessment Guidance for Superfund, Vol. 1 (USEPA 2004) and are
177 summarized in the Supplementary Material, Table S3. Because of the lack of information about
178 ABS factor of the target carcinogens, we have followed the USEPA recommendations that
179 consider that 10% of the concentration of semivolatile compounds is dermally absorbed (USEPA
180 2007).
181 Finally, we have also estimated the daily intake of nicotine by age group that was calculated as
182 the sum of the results obtained using Equations 3 and 4 for non-dietary ingestion and dermal
183 exposure, respectively (USEPA 2004).
184

$$EDI_{ingestion} = \frac{C \cdot IR}{BW} \qquad [3]$$

185

$$EDI_{dermal} = \frac{C \cdot SA \cdot AF \cdot ABS}{BW} \qquad [4]$$

186

187 For all the risk assessment calculations, nitrosamines concentrations below the LODs and the
188 LOQs were replaced with a value equal to half the LOD or half the LOQ in accordance with the
189 USEPA criteria (USEPA 2000).

190 *2.5. Statistical analyses*

191 Statistical analyses were carried out with Statgraphics- Plus 5.1 (Magnugistic, Rockville, MD,
192 USA). Because of the wide and skewed distribution of concentrations, data were log-transformed
193 prior to the statistical analyses. The transformed data followed a normal distribution. Linear

regressions and t-test were conducted to compare the medians and assess correlations between the different variables. Measurements under the LODs and LOQs were substituted with a value of one-half the LOD or the LOQ, respectively.

3. Results

3.1. Nicotine and nitrosamines in settled house dust

A summary of the concentrations of the 14 target compounds analysed in this study in house dust samples collected in the homes classified as smokers' and non-smokers' are shown in Table 3. The number of occurrences of each compound in the samples is also indicated. As expected, the total concentrations of the 14 target compounds in house dust were higher in smokers' homes than in the non-smokers' ones, with total abundances up to a factor of 60 higher, and with median concentrations around a factor of 8 higher. Nicotine, which is the main marker of tobacco smoke, was detected in all the studied samples, including those from non-smoker occupied homes, demonstrating the extent to which THS can spread beyond the source. Nicotine was the most abundant organic nitrogen target compound found in both non-smokers' and smokers' homes with median concentrations of 2.3 µg g^{-1} and 26 µg g^{-1}, respectively, and the maximum value observed was 342 µg g^{-1} in one of the smokers' dust samples.

The TSNAs studied were most frequently detected in smokers' homes dust samples (41-95%), except for NNK, which was more frequently detected in non-smokers' homes, but at much lower concentrations (median 0.54 µg g^{-1} in smokers' dust and 0.04 µg g^{-1} in non-smokers'). The most abundant TSNA was N-nitrosonatabine (NAT, max. up to 73 µg g^{-1} in smokers' dust). Although, some differences have been found in the individual concentrations, the total concentrations of the non-specific nitrosamines (NDMA, NMEA, NDEA, NDPA, NMor, NPyr, NPip and NDBA) in both kinds of samples were statistically comparable (test t, p=0.05). Among these compounds N-nitrosomethylethylamine (NMEA) was the most abundant, occurring in all the smokers' samples and in 91% of the non-smokers' samples, with median concentrations of 0.36 µg g^{-1} and 0.44 µg g^{-1} respectively (median values statistically comparable, t-test, p=0.05).

Representative chromatograms of the dust samples are shown in Figure 2 and show the increased number of different organic nitrogen compounds found in the house dust collected in a smokers' home.

To determine the influence of tobacco smoke in THS composition, we have investigated the relationship of nicotine with the number of cigarettes smoked by all occupants per day. The nicotine concentrations observed were correlated with the number of cigarettes smoked per day by the occupants inside the homes (R^2=0.859, p<0.001, Supplementary Material, Figure S1A). Furthermore, these nicotine levels also correlated with the cigarettes that the occupants smoked at locations outside their homes (R^2=0.628, p<0.001, Supplementary Material, Figure S1B). A medium degree of correlation was found between the total TSNAs concentrations and the nicotine

concentrations in house dust samples from smokers' homes ($R^2=0.466$, p<0.001, Supplementary Material, Figure S2), but this was not apparent in the non-smokers' samples ($R^2=0.028$, p>0.001). The non-specific N-nitrosamines did not correlate with nicotine concentrations in either non-smokers' ($R^2=0.04$, p>0.001) or smokers' ($R^2=0.07$, p>0.001) house dust samples, indicating that external ambient air pollution is likely the main source of these compounds.

3.2. Cancer risk assessment of THS exposure

Using the observed concentrations of the target species, cancer risk assessment was estimated for the ten carcinogenic target nitrosamines with available official toxicological data. The cumulative cancer risk through non-dietary ingestion by group age and the cumulative risk considering a lifetime exposure of 70 years, calculated using Equation 1, are shown in Table 4a. The highest calculated risks were for children from 1 to < 6 years, exposed to observed levels in house dust from smokers' homes, with a median calculated risk of 9.6×10^{-5} (9.6 additional cancer cases per 100,000 children exposed) and a maximum risk of 1.0×10^{-3} (1 additional cancer cases per 1,000 children exposed). House dust values from non-smokers' homes gave lower risk estimates, with median and maximum risk values of 3.3×10^{-5} and 1.7×10^{-4}, respectively. For the 1 to <6 years age group, the estimated risk for ALL the samples from non-smoking homes in this study exceeded the USEPA guideline of 1 excess cancer cases per 1 million population exposed (USEPA 2011). Furthermore, for a lifetime exposure, 83% of the non-smokers' and all the smokers' samples also exceeded the upper-bound excess lifetime cancer risk recommended by the WHO for carcinogens in drinking water (1×10^{-5}) (WHO 2011). The specific role of tobacco smoke in these risk estimations can be evaluated using the combined contribution of the two carcinogenic TSNAs, NNN and NNK. For children between 1 to <6 years the median and maximum ingestion risk estimated for these TSNAs were 3×10^{-5} and 9.9×10^{-4} for smokers' homes and 1.9×10^{-6} and 1.8×10^{-5} for non-smokers' homes. For this age group, the estimated risk for these TSNAs exceeded the upper-bound of 10^{-6} in 77% of the smokers' and 64% of the non-smokers' homes and the 10^{-5} threshold in 50% of the smokers', and 27% of the non-smokers' homes. The contribution of the other 3 TSNAs to the risk cannot be estimated because of the lack of toxicological data.

The calculated risk estimates, based on a lifetime exposure (0-70 years) to the individual carcinogen nitrosamines in house dust for a non-dietary ingestion pathway, are shown in Figure 3. In smokers' dust the median estimated risk of five target compounds (NDMA, NMEA, NDEA, NDBA and NNK) compounds exceeded the USEPA threshold (10^{-6}). Of these, the tobacco specific compound NNK, presented the highest contribution to the risk with a median risk over WHO guideline (10^{-5}) and a maximum over 10^{-3}. In non-smokers' samples three compounds (NMEA, NDBA and NNK) presented median risks over 10^{-6} and of these only NMEA median risk was over 10^{-5}.

Dermal absorption is another important pathway of exposure to contaminants bound to settled dust. However, this pathway is usually overlooked in risk assessment estimations. The dermal

268 exposure risks, accepting a 10% of dermal absorption value for all the carcinogen compounds
269 (USEPA 2007), as a compromise, are summarised in Table 4b. Since the estimated dermal risks
270 depend, among other factors, on the body surface, this pathway of exposure is more relevant for
271 adults. The median and maximum levels calculated for dermal exposure over a lifetime of 70
272 years were $2.1×10^{-5}$ and $2.3×10^{-4}$ in the smokers' homes and $7.3×10^{-6}$ and $3.7×10^{-5}$ in non-
273 smokers' ones. Although dermal risks estimates were generally lower than those found through
274 non-dietary ingestion, the values in most of the samples still exceeded the USEPA threshold.
275 Assuming both pathways of exposure to settled house dust contamined with THS, the cumulative
276 risks can be estimated as the sum of the non-dietary ingestion and the dermal absorption risks.
277 Assuming this lifetime exposure to both pathways, 96% of the smokers' dust samples and 83% of
278 the non-smokers' were calculated to exceed the 10^{-5} risk threshold.

279 In addition to any carcinogenic effects, chronic and acute non-carcinogenic effects may also be
280 related to THS exposure. We have also evaluated the exposure to nicotine, which was the most
281 abundant target compound in both kinds of samples (see Equations 3 and 4). The estimated daily
282 intake of nicotine by ingestion and dermal contact of THS is shown in Table 4, with a maximum
283 calculated daily intake of up to 1.73 µg per kg of body weight for children living in the smoker
284 occupied houses studied.

285 **4. Discussion**

286 Since the detection of nicotine in house dust for the first time by Hein et al. in 1991 (Hein et al.
287 1991), the contamination of residential homes with THS has been demonstrated mainly based on
288 the occurrence of nicotine, 3-ethenylpyridine and polycyclic aromatic hydrocarbons in dust, air
289 and surfaces of smokers' homes and non-smokers' homes formerly occupied by smokers (Hoh et
290 al. 2012; Matt et al. 2004; Matt et al. 2011b; Singer et al. 2003). The potential role of THS in
291 tobacco-related illnesses has been questioned however because of the poor level of
292 characterisation of the constituents of THS, as well as the lack of studies focused on human
293 exposure. Furthermore, recent studies question whether nicotine levels are representative of the
294 carcinogenic tobacco-related compounds in THS (Matt et al. 2011b). Whilst TSNAs have been
295 suspected to form part of THS as a result of laboratory studies (Sleiman et al. 2010), here, we
296 demonstrate for the first time the ubiquitous presence of carcinogenic tobacco-specific
297 compounds, such as TSNAs, in settled house dust found in a panel of smokers' and non smokers'
298 homes.

299 Comparing with previous studies the concentrations of nicotine found in the non-smokers' dust
300 samples in this study were similar to those found in a previous study in San Diego (Matt et al.
301 2011b), but lower than those reported in Baltimore (Kim et al. 2008). Here we also detected
302 TSNAs in non-smokers' homes, indicating that THS is certainly an additional pathway of exposure
303 of non-smokers to TSNAs. The lack of correlation between nicotine and TSNAs concentrations in
304 smoke-free homes would suggest that TSNAs formed in smoking environments, can then persist
305 for extended periods, possibly due to partitioning to ambient particles, and subsequently be

transported into non-smokers' homes from outside. This hypothesis would predict that urban non-smoking homes would be more exposed to external particulate matter than rural homes. Dust samples collected from urban homes in multiple occupancy buildings, such as flats and apartments, showed generally higher concentrations of TSNAs, but further research is needed to confirm this trend. In the same way, nicotine showed no clear relationship with the concentrations of the non-specific N-nitrosamines observed in non-smokers' homes, but concentrations were elevated in urban apartment homes occupied by non-smokers.

In contrast, a moderate correlation was observed between nicotine concentrations and the concentrations of TSNAs in smoker occupied homes, indicating that the majority of the TSNAs observed at these locations were the result of smoking within the home. The influence of other parameters, such as the ageing of the dust, the amount of airborne oxidants, the frequency of vacuum cleaning and ventilation could explain the weak correlation between nicotine and TSNAs observed in some samples. These parameters should be taken into account in future studies to better understand nicotine degradation in indoor environments. Although in general non-specific N-nitrosamines were higher in smokers' homes, there was not a clear correlation between these compounds and the concentrations of nicotine. This lack of correlation could be explained because of the high vapour pressures of some N-nitrosamines that tend to exist predominantly in the gas phase (Mahanama and Daisey 1996). However, other sources of atmospheric N-nitrosamines can contribute to the concentrations of N-nitrosamines in settled house dust, especially in urban and high traffic areas with high levels of pollution from combustion processes and cooking.

Another important issue addressed here is whether or not smokers who smoke only outside the home, but in close proximity, place their children at potential risk. Previous studies found that the PM_{10} and nicotine concentrations in homes, where members of the households only smoked outside, were significantly higher compared with the homes of non-smoking families (Matt et al. 2004; Rumchev et al. 2008). The strong correlation between the concentrations of nicotine that were found in the house dust from smokers' homes and the number of cigarettes smoked by the members of the household outside their homes demonstrates that tobacco smoke components are released to indoor environments by additional pathways such as off-gassing from the smokers' clothing or exhaled toxins.

The results presented here indicate that significant concentrations of N-nitrosamines and TSNAs are present in houses contaminated with cigarette smoke, however risk estimate calculations have limitations and uncertainties should be taken into account. First, there is limited available toxicological data about the target compounds. For example, the main metabolite of NNK, NNAL, does not have official toxicological data but is suspected to have the similar carcinogenicity as its precursor (Hecht 2008). Therefore, the risk of exposure to the NNAL levels observed could not be estimated. Also, most of the body weight values used for risk assessment calculations come from the USA average (USEPA 2011). Since the samples were taken in Spain and average weights

344 are lower in this country (INE 2001), the use of the USA values is probably underestimating the
345 risk exposure.

346 Additional uncertainty comes from the assumption of 10% dermal absorption for all compounds,
347 which provides only a rough approximation of the true risks of this pathway of exposure.
348 Moreover, it has to be considered that in the presence of nitrous acid the skin-bound dust nicotine
349 could react producing 0.05% NNK (Sleiman et al. 2010). According to this, the households can be
350 dermally exposed to an extra 0.16, 0.23, 0.33 and 0.44 ng of NNK per day per kg of body weight,
351 by age group, respectively.

352 Other uncertainties come from the consideration that the risks for the individual compounds are
353 cumulative, but possible mixture-related effects, such as antagonistic, synergistic, potentiating or
354 additive may occur in complex mixtures (Sterner 2010) such as THS. Because of the absence of
355 information about these mixture-related effects, we could not consider them in this study. In
356 addition, the risk estimated here has not considered other pathways of exposure such as the re-
357 suspension and inhalation of the finest particles of dust. Moreover, the re-estimation of risk by
358 replacing non-detected values with ½ LOD and non-quantified values with ½ LOQ could
359 overestimate risk, but only in less than 15% of samples (USEPA 2000). Finally, house dust
360 samples included in this study were collected using the households' vacuum cleaners in their
361 regular use. The collection of settled dust in a specific surface area of the house using a cyclone
362 vacuum cleaner would also allow the estimation of the risk by means of surface loading
363 measurements, which are usually more appropriate for human exposure assessment (Mercier et
364 al. 2011).

365 Despite the uncertainties and limitations associated with risk estimates, this study presents the
366 first clear evidence about the potential risk of exposure to nitrosamines and TSNAs, whose only
367 source is tobacco, observed in house dust. The cancer risk values estimated here demonstrate
368 that THS is a major pathway of exposure of N-nitrosamines and TSNAs, even in some non-
369 smokers' homes. Although the risk is significant for all the age groups, children between 1 and <6
370 years old are especially vulnerable to THS exposure, through accidental ingestion of settled
371 house dust and through contact of exposed surfaces followed by hand to mouth transfer. The
372 maximum risk calculated was for a home where 3 members of the household smoked, with the
373 cumulative cancer risk of exposure to levels in this house estimated as 1 additional cancer case
374 per 1000 children exposed. A recent report of the WHO estimated that 40% of children are
375 exposed to second hand smoke (Oberg et al. 2011). However, this may be an underestimate of
376 the impact of smoking on children and the number and type of exposure should be revised
377 according with the risk levels found in THS here. We have demonstrated that house dust in some
378 non-smoker occupied homes contained chemical tracers of THS. The cancer risk for children,
379 through ingestion of settled house dust contaminated with NNN and NNK, exceeded the USEPA
380 recommended threshold in 64% of the dust samples collected in non-smokers' homes. Settled
381 house dust has already been estimated to be the major route of exposure of children to lead and
382 some persistent organic pollutants (Ott et al. 2007). Besides, the estimated daily intake of nicotine

383 may cause chronic health effects and potentially nicotine-addiction in non-smokers, including
384 children (IARC 2004).

385 **5. Conclusions**

386 In this study, we have determined the presence of 14 tobacco-related organic nitrogen
387 compounds in settled house dust samples from smokers' and non-smokers' homes. Our study
388 demonstrates for the first time the widespread presence of tobacco related carcinogens in house
389 dust, even in "smoke free" environments. Cancer risk assessment of the carcinogen compounds
390 showed that settled dust is a major route of exposure to TSNAs in children and non-smokers who
391 are not directly exposed to secondhand smoke. Hence, the risk of exposure of non-smokers to
392 tobacco through inadvertent ingestion and dermal exposure of thirdhand smoke should not be
393 overlooked, and its impact included in future educational programs and tobacco-related public
394 health policies.

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509 | **Tables**

510 **Table 1.** IARC classification and oral slope factors of target compounds included in our

511 study, and the source of this information.

Nitrosamine	IARC classification[a]	Oral slope factor
N-nitrosodimethylamine (NDMA)	2A	51[b]
N-nitrosomethylethylamine (NMEA)	2B	22[b]
N-nitrosodiethylamine (NDEA)	2A	150[b]
N-nitrosodi-n-propylamine (NDPA)	2B	7[b]
N-nitrosomorpholine (NMor)	2B	6.7
N-nitrosopyrrolidine (NPyr)	2B	2.1[b]
N-nitrosopiperidine (NPip)	2B	9.4[c]
N-nitrosodi-n-butylamine (NDBA)	2B	5.4[b]
Nicotine	-	-
N'-nitrosonornicotine (NNN)	1	1.4[c]
N'-nitrosoanatabine (NAT)	3	-
N'-nitrosoanabasine (NAB)	3	-
4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK)	1	49[c]
4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanol (NNAL)	-	-

512 [a] IARC classifications: group 1, carcinogen to humans; group 2A, possible carcinogen to humans; group 2B,
513 probably carcinogen to humans; group 3, not classifiable as to its carcinogenicity to humans (IARC, 2013).
514 [b] Data from IRIS (IRIS, 2013)
515 [c] Data from OEHHA (OEHHA, 2007)

516 **Table 2.** Characteristics of the homes and the households included in this study

Characteristics	Smokers' homes (n=22)	Non-smokers' homes (n=24)
Home		
Location	Urban: 81%	Urban: 87%
	Suburban: 19%	Suburban: 13%
	Low to moderate traffic	Low to moderate traffic
Building information	Age*: 12	Age*: 18
	Flat: 81%	Flat: 87%
	House: 19%	House: 13%
	Fireplace: 0%	Fireplace: 0%
	Carpeted floor: 0%	Carpeted floor: 0%
Households information		
Kind of residents	Adults*: 2	Adults*: 2
	Homes with children: 27%	Homes with children: 37%
	No. Children: 1 or 2	No. Children: 1 or 2
	Ages*: 7	Ages*: 6
Pets	None: 82%	None: 79%
	One: 18%	One: 21%
Smokers per home	From 1 to 3	
Number of cigarettes per day*	Total smoked cigarettes: 17	-
	Cigarettes smoked indoors: 5	-
Household products		
Use of incense or candles	14% of the homes	8% of the homes
	Frequency*: 1/week	Frequency*: 1/week
Cleaning information		
Vacuum frequency*:	1.5/week	1.5/week
Ventilation frequency:	Everyday 54%	Everyday: 50%
	Twice a week: 32%	Twice a week: 42%
	Once a week: 9%	Once a week: 8%

517 * Median values

518 **Table 3.** Concentrations of the target compounds in the settled house dust samples (µg g^{-1}).

519 %Quant. indicates the samples in which the target species were above the LOQ.

Compound	Smoker's house dust (µg g^{-1}, n=22)						Non-smoker's house dust (µg g^{-1}, n=24)					
	Min	0.25	Median	0.75	Max	% Quant.	Min	0.25	Median	0.75	Max	% Quant.
NDMA	n.d.	0.01	0.01	0.31	3.9	45	n.d.	0.003	0.003	0.01	2.0	9
NMEA	0.02	0.20	0.36	0.60	1.6	100	n.d.	0.22	0.44	1.1	3.2	91
NDEA	n.d.	0.002	0.04	0.15	1.2	59	n.d.	0.002	0.01	0.03	0.39	35
NDPA	n.d.	0.001	0.003	0.005	0.03	9	n.d.	n.d.	n.d.	n.d.	<LOQ	0
Nmor	n.d.	0.003	0.01	0.01	1.9	36	n.d.	0.002	0.01	0.01	0.08	22
Npyr	n.d.	0.002	0.003	0.01	0.27	14	n.d.	0.002	0.002	0.01	0.05	13
Npip	n.d.	0.002	0.01	0.04	0.73	50	n.d.	0.002	0.002	0.01	0.07	22
NDBA	n.d.	0.04	0.10	0.23	0.54	91	n.d.	0.03	0.07	0.12	0.37	83
Nicotine	4.33	17	26	62	342	100	0.62	1.5	2.3	3.3	5.3	100
NNN	n.d.	0.004	0.02	0.20	1.8	41	n.d.	0.004	0.004	0.02	0.05	22
NNT	n.d.	0.003	0.07	2.7	73	55	n.d.	0.003	0.01	0.03	1.5	26
NNB	n.d.	0.07	0.51	1.8	13	82	n.d.	0.003	0.00	0.01	0.03	9
NNK	n.d.	0.02	0.54	1.6	20	68	n.d.	0.02	0.04	0.06	0.37	74
NNAL	n.d.	0.15	0.46	1.4	16	95	n.d.	0.01	0.03	0.06	1.3	39
Total	6.6	21	31	90	426		1.4	3	4	4.9	6.8	

520 **Table 4a.** Cancer risk estimations for the **non-dietary ingestion** of settled house dust, by
521 age group, expressed in number of calculated excess cancer cases per exposed population.

522

Age range (years)	Smokers'					Non-smokers'				
	Min	25%	Median	75%	Max	Min	25%	Median	75%	Max
Birth to <1	3.7×10^{-6}	1.6×10^{-5}	4.0×10^{-5}	8.3×10^{-5}	4.3×10^{-4}	8.8×10^{-7}	5.9×10^{-6}	1.4×10^{-5}	2.0×10^{-5}	7.0×10^{-5}
1 to <6	9.0×10^{-6}	3.9×10^{-5}	9.6×10^{-5}	2.0×10^{-4}	1.0×10^{-3}	2.1×10^{-6}	1.4×10^{-5}	3.3×10^{-5}	4.9×10^{-5}	1.7×10^{-4}
6 to < 21	3.0×10^{-6}	1.3×10^{-5}	3.2×10^{-5}	6.6×10^{-5}	3.4×10^{-4}	7.0×10^{-7}	4.8×10^{-6}	1.1×10^{-5}	1.6×10^{-5}	5.6×10^{-5}
21 to 70	1.7×10^{-6}	7.6×10^{-6}	1.9×10^{-5}	3.9×10^{-5}	2.0×10^{-4}	4.1×10^{-8}	2.8×10^{-6}	6.4×10^{-6}	9.5×10^{-6}	3.3×10^{-5}
Birth to 70	1.7×10^{-5}	7.5×10^{-5}	1.9×10^{-4}	3.9×10^{-4}	2.0×10^{-3}	4.1×10^{-6}	2.8×10^{-5}	6.4×10^{-5}	9.5×10^{-5}	3.3×10^{-4}

523 **Table 4b.** Cancer risk estimations for the **dermal exposure** to settled house dust, by age
524 group, expressed in number of cases per exposed population.

Age range (years)	Smokers'					Non-smokers'				
	Min	25%	Median	75%	Max	Min	25%	Median	75%	Max
Birth to <1	9.3×10^{-8}	4.1×10^{-7}	1.0×10^{-6}	2.1×10^{-6}	1.1×10^{-5}	2.2×10^{-8}	1.5×10^{-7}	3.5×10^{-7}	5.1×10^{-7}	1.8×10^{-6}
1 to <6	3.2×10^{-7}	1.4×10^{-6}	3.5×10^{-6}	7.3×10^{-6}	3.8×10^{-5}	7.7×10^{-8}	5.3×10^{-7}	1.2×10^{-6}	1.8×10^{-6}	6.1×10^{-6}
6 to < 21	5.1×10^{-7}	2.2×10^{-6}	5.5×10^{-6}	1.1×10^{-5}	5.9×10^{-5}	1.2×10^{-7}	8.3×10^{-7}	1.9×10^{-6}	2.8×10^{-6}	9.6×10^{-6}
21 to 70	1.0×10^{-6}	4.6×10^{-6}	1.1×10^{-5}	2.4×10^{-5}	1.2×10^{-4}	2.5×10^{-7}	1.7×10^{-6}	3.9×10^{-6}	5.7×10^{-6}	2.0×10^{-5}
Birth to 70	2.0×10^{-6}	8.6×10^{-6}	2.1×10^{-5}	4.4×10^{-5}	2.3×10^{-4}	4.7×10^{-7}	3.2×10^{-6}	7.3×10^{-6}	1.1×10^{-5}	3.7×10^{-5}

525 **Table 5.** Estimated daily intake of nicotine by non-dietary ingestion and dermal exposure,
526 expressed in ng per kg of body weight per day.

Age range (years)	Smokers'					Non-smokers'				
	Min	25%	Median	75%	Max	Min	25%	Median	75%	Max
Birth to <1	21	95	129	307	1637	3.0	6.6	11	16	25
1 to <6	22	100	136	325	1729	3.1	6.9	12	17	27
6 to < 21	13	61	83	197	1048	1.9	4.2	7.1	10	16
21 to 70	13	60	81	193	1030	1.9	4.1	7.0	10	16

527 **Figure captions**

528 **Figure 1.** Structures and formation pathways of the main tobacco specific N-nitrosamines

529 (TSNAs).

530 **Figure 2.** GC×GC-NCD chromatograms of smokers' (A) and non-smokers' settled house dust

531 (B).

532 **Figure 3.** Percentile distribution of the LCRs of the carcinogen nitrosamines, in smokers' (a)

533 and non-smokers' settled house dust (b). The box plot of each carcinogen nitrosamines

534 represents the 25th and 75th percentile of the LCRs and the horizontal line inside the box

535 indicates the median LCR. The bottom and the top lines indicate the minimum and the

536 maximum LCRs, and the circle symbols the average LCR. The horizontal red line indicates

537 the threshold risk recommended by USEPA (10^{-6}).