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SECONDHAND SMOKE EXPOSURE IN SCHOOL CHILDREN IN MALTA ASSESSED THROUGH URINARY BIOMARKERS

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Abstract

School children may be exposed to secondhand smoke (SHS) either at home, in transit or in social gatherings permitting smoking in their presence. Questionnaires about SHS often underestimate prevalence and extent of exposure. A more accurate tool is the use of biomarkers such as cotinine (COT) and trans-3'-hydrocycotinine (3HC) as biomarkers of SHS exposure, alongside 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), a reduction product in the body of the tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), both potent carcinogens. We measured urinary COT, 3HC and total NNAL using sensitive and specific high-performance LC-MS/MS methods. The limit of quantification (LOQ) for each assay were 0.05 ng/mL, 0.1 ng/mL and 0.25 pg/mL respectively. The aim of this study was to evaluate the exposure to SHS of school children (9–11 years), from five public schools in the island of Malta, from questionnaire information about smoking at home and verify it by urinary biomarker data of COT, 3HC and NNAL. These biomarkers were measurable in 99.4%, 95.4% and 98.3% of the participating children respectively. From the children reporting smoking at home, 11% had a history of asthma and had COT, 3HC and NNAL geometric mean concentrations double compared to the non-asthmatic group. In has been confirmed that non-smokers exposed to SHS and THS have a higher NNAL/COT ratio than the group identified as smokers according to specific and defined COT threshold levels (despite the fact that a priori, the entire study group was composed

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CRediT authorship contribution statement

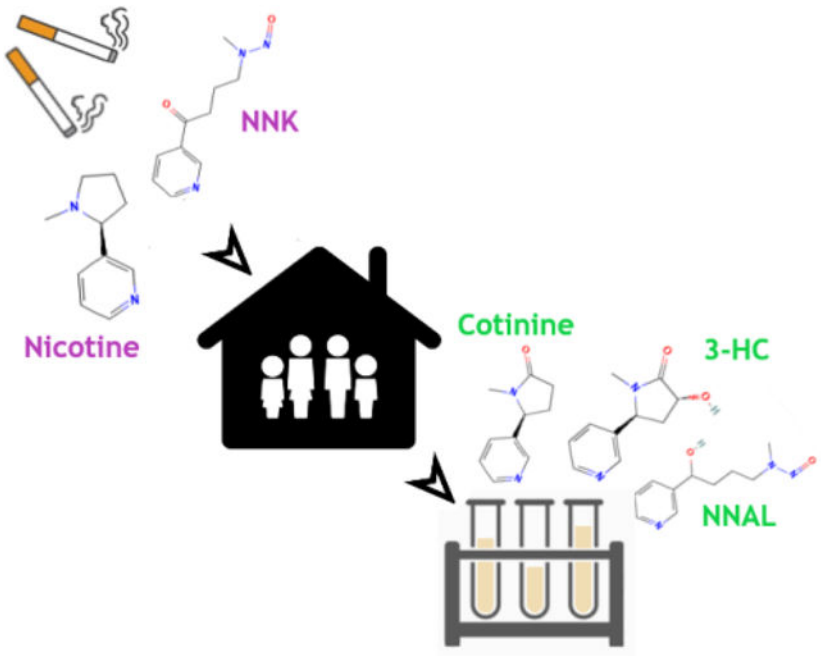
Noel J. Aquilina: Conceptualization, Sample acquistion, Methodology, Writing - original draft.
Neal L. Benowitz: Supervision, Writing - review & editing.
Peyton Jacob III: Supervision, Writing - review & editing.
Peter Fsadni: Sample acquistion, Writing - review & editing.
Stephen Montefort: Sample acquistion, Writing - review & editing.
All authors contributed to this paper and approved its content.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

of non-smokers). The implication of high measured levels of urinary NNAL in children should be of concern given its potency. A main effects multifactor ANOVA model was developed and the children's house and school locations and the smoking frequency were statistically significant to predict the levels of the three metabolites. For 3HC only, the status of the employment of the mother was also an important predictor.

Graphical Abstract



Keywords

Secondhand Smoke; Thirdhand Smoke; Cotinine; trans-3'-hydroxycotinine; NNAL; school children

Introduction

A central policy of the Framework Convention on Tobacco Control by the World Health Organisation is encouragement of no smoking in public areas and indoors because secondhand smoke (SHS) is strongly associated with several medical conditions and death (You et al., 2021). There seems to be no safe or risk-free level of SHS exposure (Center for Disease Control and Prevention, 2010; Homa et al., 2015; U.S. Department of Health and Human Services, 2014). Due to exposure to SHS, infants could suffer from the sudden infant death syndrome (Homa et al., 2015), children younger than five years have a greater risk of lower respiratory infections, asthma induction and exacerbation (Öberg et al., 2011), alterations in lung development, otitis media and chronic middle ear effusions (NCI, 1999) and an increased probability of becoming a cigarette smoker (Treyster and Gitterman, 2011). The International Agency for Research on Cancer, amongst other groups, have classified SHS as a human carcinogen. More than 50 epidemiologic studies have shown that SHS

causes lung cancer in non-smokers (IARC, 2004), but the risk of future lung cancer from SHS exposure in children is poorly understood. Lack of an accurate exposure assessment during childhood is one of several factors that hinders the investigation of this relationship (Hecht et al., 2006).

The overall children's exposure to tobacco related pollutants in the indoor microenvironment comes from both fresh and ageing SHS, primarily through inhalation but also by dermal absorption and ingestion. Especially in reduced ventilation environments, as ageing SHS dissipates it contaminates the microenvironment further, now termed Thirdhand Smoke (THS) (Jacob et al., 2017; Matt et al., 2011). Although THS is linked to low levels of pollutants, it can lead to a longer term exposure, and thus the possible adverse health effects of THS in children are of concern. In vitro and animal studies demonstrate that THS is cytotoxic and can affect several human organs (Martins-Green et al., 2014).

Evaluating the exposure of children to active smoking and SHS through questionnaires, generally filled by their parents, is often misleading and inaccurate (Benowitz et al., 2016; Goniewicz et al., 2011). The link between the questionnaire information and the actual uptake dose of tobacco-specific pollutants is very much dependent on the proximity to the source, its strength and the dynamics of SHS over time (Benowitz, 1996; Joseph et al., 2005). Initially biochemical verification was used to distinguish between smokers and non-smokers, but nowadays due to better analytical capabilities, assays are able to monitor even low-level exposure by non-smokers (Benowitz et al., 2020; Hecht, 2002; Hecht et al., 2006).

Two metabolites derived from nicotine found in SHS are cotinine (COT) and (3'R,5'S)-trans-3'-hydroxycotinine (3HC). Urinary COT and 3HC have been extensively used as a marker for SHS exposure (Avila-Tang et al., 2013; Kim et al., 2016) as the former shows high specificity while the latter is a more sensitive biomarker for low-level exposure (Jacob 3rd et al., 2011) . 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), is the prevalent tobacco-specific nitrosamine (TSNA) and a potent carcinogen known to cause tumors in rodents (Hecht, 2003, 1999; Hecht et al., 2013, 2006, 2001; Hecht and Hoffmann, 1988) (Hecht, 2003, 1999; Hecht et al., 2013, 2006, 2001; Hecht and Hoffmann, 1988). Evidence supports a significant role of NNK as a cause of lung cancer in smokers and nonsmokers exposed to SHS (Hecht, 1999; IARC, 2007). 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) is the primary metabolite of NNK and the main TSNA measured in urine (Kozlovich et al., 2015; Xia et al., 2020; Yuan et al., 2014). NNAL is also carcinogenic (Hecht, 2008). Compared to COT, NNAL has a longer half-life and it appears to be a useful biomarker to represent a longer term exposure to SHS and THS (Benowitz et al., 2020; Goniewicz et al., 2011; Jacob et al., 2013).

As of 2017, the adult daily smoking prevalence in Malta is on average 20% (World Health Organization, 2019). Although according to the Country Health Profile of the European Commission for Malta for 2019 (European Commission, 2019), the smoking rates among 15 and 16-year-olds have fallen substantially in the last 10 years. According to the 6th Edition of the Tobacco Atlas, based on 2015 data, about 2.54% of boys and 3.03% of girls of age less than 15 years, do smoke (Cahn et al., 2018).

A limited number of studies have addressed the exposure of children to SHS and TSNA through the use of biomarkers (Dempsey et al., 2012; Hecht et al., 2006, 2001; Hertsgaard et al., 2008; Jeong et al., 2021; Kassem et al., 2014; Stepanov et al., 2006; Thomas et al., 2011; Vogel et al., 2011). Studies in Malta carried out in 2001, based on questionnaire data only, reported that 31% and 51% of children of age groups (5–8 years) and (13–15 years) respectively, were exposed to SHS. (Montefort et al., 2012). Ours is the first study to evaluate the levels of exposure to SHS and THS of young children (9–11 years old) in Malta, through the use of questionnaire data and verified by urinary biomarkers for nicotine and NNK.

Experimental

1. Study Population—Five public schools in Malta were randomly chosen to participate in a European project called “Schools Indoor Pollution and Health Observatory Network in Europe” (SINPHONIE) between October 2011 and February 2012. This was a project looking into how air quality in schools effects respiratory conditions in school children across Europe (Csobod et al., 2014; Fsadni et al., 2015). 174 students participating in this study were in the 9–11-year age group. For logistical reasons, the schools were all chosen from Malta which for statistical purposes was subdivided in five districts (Figure 1). The Northern Harbour district is the most densely populated and two schools were from this district (S1, S2). The Southern Harbour district is the second most populated and one school represented this district (S3). The South Eastern and the Western districts follow by number of inhabitants and one school from them was chosen, (S4 and S5). The schools S1 and S2 are representative of highly urbanised areas, S3 is situated in an urban-background area, S4 close to a port/harbour and industrial area while S5 can be considered to be in an urban-background/rural setting.

This study was approved by the University of Malta Research Ethics Committee and the Education Department Research Directorate. Both students and their parents were provided with written information sheets about the study, and a meeting for all students and parents was held at each school. Parents were asked to sign a consent form. Standardized health questionnaires based on the International Study of Asthma and Allergies in Childhood (ISAAC)(Asher et al., 1995) were answered by the children’s parents. The questions pertaining to exposure to SHS were structured to identify if the students were exposed to SHS at home, what was the frequency of exposure, how many smokers were around and how many cigarettes were smoked in a determined period of time. Information on the clinical diagnosis of asthma in the students was also recorded.

2. Urine Sampling Protocol, Storage and Analysis—The participants were asked to provide a morning void urine sample in a labelled polypropylene bottle. 30 mL of each sample were pipetted in a labelled, cryogenic polypropylene tube and shipped over dry ice to the Clinical Pharmacology Laboratory in the Division of Cardiology, Department of Medicine, University of California San Francisco (UCSF), USA and stored at 20 °C until analyses.

The three metabolites were extracted and analysed using well established methods. Total NNAL concentrations were measured by liquid chromatography – tandem mass spectrometry (LC-MS/MS) as described by Jacob et al. (Jacob et al., 2008). COT and 3HC were measured by LC-MS/MS (Jacob et al., 2011)(). The LOQ for the COT, 3HC and NNAL assays were 0.05 ng/mL, 0.1 ng/mL and 0.25 pg/mL respectively.

3. Methods

The biomarker verification of the exposure to SHS will be based on COT threshold levels due to its high sensitivity and specificity (Benowitz, 1999). These threshold levels, as defined by Benowitz et al., (2016) classify the study in four groups. COT concentrations less than 0.05 ng/mL (COT<LOQ) would be considered as the unexposed group. The remaining will be the exposed group, subdivided in three categories, namely 0.05–0.25 ng/mL indicating low exposure to light SHS and possibly also to THS, 0.25–30 ng/mL, typical of a medium exposure defined as normal exposure to SHS or occasional tobacco use and >30 ng/mL, high concentrations, generally associated with active smoking. 3HC is considered a more sensitive biomarker for low-level exposure (Jacob 3rd et al., 2011) and thus will be reported for each exposure category defined above. Due to its relatively long half-life of 40–45 days, NNAL is a suitable indicator of the long term exposure or when there is a delay between exposure and biomarker measurement (Goniewicz et al., 2011). Goniewicz et al., (2011) found that the high correlation between NNAL and COT makes the NNAL/COT ratio very suitable to evaluate the exposure to NNK in relation to ageing SHS and THS and thus will be reported in this study.

As a spot urine sample was taken, metabolites are typically corrected for creatinine, however according to (Allen et al., 2004) there would be an important objection to report any of the metabolites as analyte/creatinine ratio in children. Children who are growing rapidly have developing musculature and excrete creatinine at a much lower daily rate per kg body mass than adults. This would make their standardized ratio much higher relative to an adult for equal concentrations. This objection could be avoided if all such analyte/creatinine ratios were multiplied by the subject's individualized estimate of creatinine production (mg creatinine/kg body mass/day). This individual body mass information was not available and for this reason this study will not report creatinine corrected biomarker data.

4. Statistics

All statistical analysis was carried out with SPSS version 27 (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp). To examine the association of sex, school attended and asthma diagnosis we tested differences in the COT class detection frequency with the chi-squared test. To identify if there was significant difference in metabolite mean rank across the different schools a Mann-Whitney U test was performed. A Kruskal–Wallis H test was run to evaluate whether absolute measured values of COT, 3HC and NNAL differed across covariates.

A Receiver Operating Characteristic (ROC) curve is a graph used to show performance of classification at different thresholds by plotting the true positive rate vs the false positive rate

at different classification thresholds. Lowering the classification threshold classifies more items as positive, thus increasing both False Positives and True Positives. An algorithm called the Area Under Curve (AUC) measures how well predictions are ranked and will efficiently identify a cut-off point with the highest specificity and sensitivity possible.

As the COT, 3HC and NNAL data are not normally distributed, a main effects multifactor ANOVA model is being applied as none of the predictors (associated with socioeconomic factors and smoking status at home, all collected from the parents' questionnaire) are covariates, and no interaction terms are considered. The transformation on the response variable is done so that normality of the errors is achieved. The models with the highest adjusted R^2 will be chosen, however an additional constraint will be made where a condition index higher than 10 for the predictors is not accepted even with a better adjusted R^2. This was done to avoid multicollinearity, and as a result to make the model coefficients interpretable.

5. Results

5.1. Analytical

Of 174 children, 4.6% were defined as unexposed (COT level <LOQ) and 95.4% were exposed to SHS, as defined by a COT level ≥0.05 ng/mL. 95.4% were exposed to nicotine through detection of COT in urine and verified through the quantification of 3HC (detected in 99.4% of the children. Further exposure to SHS (and NNK) was exhibited by 98.3% of the children. The number frequency distributions of the low and medium exposed group based on the COT levels described above are illustrated in Figure 2(a, b) and their corresponding NNAL distributions are shown in Figure 2(c, d). The highest exposed group was made up of 3 subjects. The characteristics of the different SHS exposed categories, inclusive of the biomarker results are presented in Table 1.

Based on the COT level subdivisions shown in Table 1, although 4.6% of the children were considered unexposed to SHS, it is not uncommon that both 3HC and NNAL were detected. The former is typically found in levels about 3–4 fold higher than COT in urine (Jacob et al., 2011) while the latter could be an indicator of THS exposure. Whilst 23.0% of the children were exposed to very low levels of SHS and THS, through COT, 3HC and NNAL levels, the majority of children, 70.7% were exposed to significant SHS or possibly used tobacco intermittently. Three children (1.7%) could be classified as active smokers.

From Table 1, for the study group (N=174), the COT geometric mean (0.9 ng/mL) indicates that most of the children are exposed to low levels of SHS. Based on the number frequency distributions in Figure 2(a), the largest subgroup (N=123) represents the medium level COT exposure with an interquartile range of 3.3 ng/mL (Table 1). A similar pattern is observed for the NNAL number frequency distributions shown in Figure 2(b), where the interquartile range of NNAL for the medium exposed group is 20.2 ng/mL. As stated earlier, for all exposure categories, 3HC concentrations are largely higher than COT levels.

Although the high exposure category is represented by a very limited number of cases showing a high variability in concentration, as expected for smokers, the concentration of the three biomarkers are appreciably higher than the other categories.

For the medium COT exposed group a ROC curve was run based on the question if children were exposed to smoking or not at home. From an ROC analysis of the NNAL data, the area under the curve was 0.754, the sensitivity was 0.649; 1-Specificity was 0.194 and the Youden Index was 0.455. Although sensitivity and specificity were not excellent, the optimal NNAL cutoff point worked out to be 9.9 ng/mL to distinguish between intermittent active tobacco use and substantial exposure to SHS. From Table 1, the medium COT subgroup showed higher variability in the NNAL levels (IQR=20.2) compared to the other subgroups.

With reference to Figure 3(a), the correlation between urinary COT and NNAL does not appear to be particularly strong (R^2=0.134) when considering the low exposure group whilst a stronger correlation (R^2=0.664), between NNAL and COT was obtained when a medium level of COT was considered (Figure 3(b)). This correlation is similar to previous studies (Benowitz et al., 2018; Bernert et al., 2010; Hecht et al., 2001; Wei et al., 2016).

When comparing the concentrations of the biomarkers in children from the different schools in Figure 4, children in schools S1 and S4 had the highest median concentrations for the three metabolites but also the highest variability. Across the schools, the median NNAL/COT ratio varied from 4.1×10^{-3} (for S3) to 7.4×10^{-3} (for S2). Further information about the metabolites from children associated with their school and hence the environment where they live is presented in Table SD1 in the Supplementary Data.

The questionnaire described in Section 1 revealed information if children were clinically diagnosed as asthmatic or not. Table SD2 describes the biomarkers' characteristics by sex and asthma diagnosis. Females and the asthmatic group exhibited a higher geometric mean concentration and interquartile range the three metabolites, however the NNAL/COT ratio was less.

77% of the study group (or 81% of the exposed children; N=134) had their parents answering the questionnaire mentioned in Section 1 in relation to the smoking status at home and if their children were clinically diagnosed as asthmatic. The biomarkers' data associated with these sub-groups is reported in Table 2.

Consistently, for the non-asthmatic sub-group, if smoking was reported to occur at home (16.4%), for the three biomarkers, their concentration was about 2.5–3 times higher than those who did not report any smoking (54.5%). Nevertheless the geometric mean and the interquartile range of the NNAL/COT ratio were relatively similar for the two sub-groups. For the asthmatic group, the sub-group reporting smoking at home (11.2%) had a substantial higher geometric mean concentration and interquartile range for all metabolites compared to the subgroup not reporting smoking at home (17.9%). For the NNAL/COT ratio the level is almost the same for both groups.The children diagnosed with asthma had COT and NNAL levels, 6 times and 3HC levels 5 times, higher than the asthmatic group that reported no SHS exposure at home.

Data for the three metabolites for subgroups of children according to socioeconomic and smoking at home characteristics gathered from the questionnaire are presented in Table SD3. It is clear that the education level of the parents, their employment status, if they receive state benefits or not has some effect, where lack of education and unemployment are conducive to higher exposure to SHS and hence to higher concentrations of the three metabolites extracted from their children's urine. The smoking status at home is also a contributor to higher metabolite levels, with increased smoking frequency and number of smokers and cigarettes smoked.

5.2. Statistics

The chi-square test showed that there is a significant association between sex and COT class ($p=0.005$) whilst no association with the school the children attended ($p=0.202$) or their asthma diagnosis ($p=0.166$).

A Kruskal-Wallis H test revealed insignificant differences ($p>0.05$) in the metabolite concentration for children with different asthmatic conditions. Significant differences ($p<0.05$) were noticed for the three metabolites when considering smoking status at home, sex and different schools.

The Mann-Whitney U test was run to identify if there was significant difference in the three metabolites mean rank across the asthmatic and non-asthmatic group when both groups reported their smoking status at home (Table 2). For the asthmatic group, for the three metabolites, the test revealed significant differences ($p<0.05$) between the group that reported smoking as compared to those who reported no exposure at smoke (Figure SD1). The same significant difference was noted for all metabolites between the non-asthmatic group that reported smoking at home as compared to the ones who reported no smoking (Figure SD2).

The Mann-Whitney U test was performed to identify if there was significant difference in metabolite mean rank across the different schools, for the three metabolites, the test revealed significant differences between school 1 and 2, 3 and 5 but not with school 4. There were insignificant differences when comparing the other schools between them. The same test was run for the group who are not exposed at home as compared to those who are exposed at home. As expected, the group who are exposed to SHS at home showed a statistically significant difference in the ranking of the three metabolites when compared to those who are not exposed. When it comes to the difference in exposure associated with sex, females also had a statistically significant higher mean rank for the three metabolites compared to males (Table SD2).

5.3. Predictive Model

81.6% (142) of the participants had the questions associated with tobacco use and socioeconomic information answered by their parents, possibly explaining the variance of the dependent variables COT, 3HC and NNAL. The socioeconomic predictors considered were the school attended (School: 1=Qormi; 2=Pembroke; 3=Fgura; 4=Birzebbugia; 5=Dingli), house location (0=clean; 1=reasonable traffic; 2=trafficked area), if the family receives state benefits (0=No; 1=Sometimes; 2=Regularly); education status of parents

(0=unfinished primary; 1=finished primary; 2=vocational; 3=finished; 4=University); and employment status of parents (0=employed; 1=part-time employed; 2=unemployed; 3=pensioner; 4=disabled). The smoking related predictors considered were smoking status at home (0=No; 1=Yes), smoking frequency at home (0=Never; 1= Sometimes; 2=Daily), number of smokers at home (0=None; 1=two smokers; 2=more than three smokers) and number of cigarettes smoked (0=None; 1=14 cigarette per day; 2=more than 5 cigarettes per day). Based on the procedure described in Section 4, the parameter estimates (given in detail Supplementary Data in Figure SD3) equations are summarized in Table 3 for the COT, 3HC and NNAL. The predictive equations for the metabolites would be the inverse natural logarithmic values of the equations in Table 3.

6. Discussion

As COT has a relatively short half-life (16 hours) (Benowitz et al., 2009), it might be suitable biomarker for the short-term, fresh SHS. As SHS ages, nicotine levels decrease quickly due to the adsorption of nicotine on surfaces such as walls and carpets and thus the inhaled dose is less, giving lower COT levels (Goniewicz et al., 2011). On the other hand it was shown that NNK levels increase over time due to the reactivity of nicotine in an indoor microenvironment with nitrous acid (Schick and Glantz, 2007; Sleiman et al., 2010). For these reasons, COT levels would represent an underestimation of the exposure to NNK (Benowitz et al., 2010). It is also more likely that the NNAL/COT ratio is more indicative of the ageing SHS or TSNA exposure (Goniewicz et al., 2011).

The NNAL cutoff value obtained in this study is in line with that obtained for active smokers by Benowitz et al. (Benowitz et al., 2018) and supports its use as a “gold standard” for active tobacco use.

For the three metabolites, insignificant difference between schools 1 and 4 could be attributed to comparable socioeconomic characteristics of these two areas and thus the smoking patterns that showed that there was no difference in the metabolites based on sex, albeit their study involved adolescents not children. Differences were observed between infants and adolescents’ concentrations of COT and NNAL, probably attributing such differences due to developmental or environmental factors that influence the metabolism of nicotine and NNK but the understanding of the mechanisms is still not supported by any data (Hecht et al., 2006). However one cannot exclude the fact that infants are closer to smokers at home most of the time unlike adolescents who would be more exposed when away from home.

In general collecting 24-hour urine samples would be ideal to represent the total daily exposure to SHS. If this is not possible and a morning, void sample is collected, the biomarkers detected due to very sensitive assays would still verify even low exposures to SHS, reported in the questionnaire in a more accurate way.

The mean COT level in children in Malta, categorized as low-level exposure was 0.1 ng/mL which is higher than what was reported for Moldovan children 0.008 ng/mL and for other studies involving relatively unexposed children in the United States, Italy and

Sweden reported by Stepanov et al., (2006) where COT concentrations ranged from 0.02–0.03 ng/mL and 0.2–0.4 ng/mL in Korean children (Jeong et al., 2021).

As 3HC is a metabolite of COT and it is claimed that it is found in higher concentrations in urine than COT and makes it a very suitable indicator for low SHS exposure (Benowitz and Jacob, 2001; Jacob III et al., 2011). All results presented in Tables 1, 2, SD1-SD3 are supportive of the abovementioned observation.

Analysing data according to the three pre-defined COT categories, for the low and intermittent SHS exposure the geometric mean concentrations obtained were very similar to the study by Benowitz et al., (2018), unlike the high exposure category where the mean was much lower. Most probably as in this study younger children were observed, their main SHS exposure pathway would be at home unlike adolescents who would have a more active lifestyle. According to Benowitz et al., (2018), the NNAL/COT ratio is higher in people exposed to SHS compared with active smokers. Although our study group was not intended to study smokers, a sub-group were identified as probably smokers from their COT urinary level and their NNAL/COT ratio. When discussing the dynamics of indoor ageing SHS, given that nicotine is rapidly adsorbed to surfaces and NNK forms during the SHS aging (Jacob et al., 2017), the NNK/nicotine ratio would increase with time. This implies that non-smokers would inhale aged SHS that would result in a higher urine NNAL/COT ratio compared with active smokers inhaling mainstream smoke.

Other studies in adults reported mean total NNAL urinary levels ranging from 5.4 to 16.3 pg/mL (Goniewicz et al., 2011; Stepanov et al., 2006). Two studies with children (Hecht et al., 2006; Stepanov et al., 2006) and another one with non-smoking adolescents (Benowitz et al., 2010) had mean levels of 15.2, 24.5 and 1.2 pg/mL urinary NNAL respectively. In this study, the geometric mean NNAL for the whole cohort was 5.1 pg/mL, certainly biased by some samples that are considered as smokers. On the other hand, if the low and medium exposed groups only are considered, the geometric mean NNAL levels were 1.12 and 8.00 pg/mL respectively. The geometric mean NNAL concentrations obtained are in line with the values obtained for children in the abovementioned studies. According to a study by Kaufmann et al. (2010) apparently children tend to have higher urinary NNAL because of higher exposure to SHS compared to adults.

In this study it appears that clinically diagnosed asthmatic students are exposed to higher levels of SHS in comparison to the non-asthmatic group, although the difference is not statistically significant. However within the asthmatic group, for the three metabolites, there are significant differences between the group that reported smoking as compared to those who reported no exposure at home.

7. Conclusion

Some limitations were identified, namely in the sample size and thus providing limited generalizability. The assessment of smoking behaviour from the questionnaire data was restricted, especially to identify intermittent smoking exposure or to provide information on any exposure occurring away from home.

To our knowledge, a very limited number of studies providing biomarker verification of exposure to SHS, primarily to nicotine and NNK, through their urinary metabolites (COT, 3HC and NNK) were reported in school children in a Mediterranean country. It is not surprising that most of the students (>95%) were exposed to SHS. In Malta smoking prevalence in adults is still relatively high.

Some areas in Malta where due to socioeconomic reasons, some parents persist to smoke at home, higher incidence of asthma in children has been reported (Montefort et al., 2009). The typically mild weather all year round in Malta, a characteristic of the Mediterranean region, is generally conducive to children accompanying adults in several social activities where smoking is still permitted both indoors and outdoors.

This argumentation has been further confirmed from the modelling exercise, where for COT and NNAL the main predictors to explain exposure to SHS were the school attended, the house location and the smoking frequency at home. For 3HC, the same predictors were important and also the status of employment of the mother. In Malta it is normal for houses near trafficked areas to be kept more closed, and thus reduced ventilation would lead to an increase in the metabolite levels as the models indicate. It is also customary that parents walk or drive their children to their school and parents tend to gather with kids after school, most probably that is why the school turned out to be an important factor for predicting the metabolite levels. It is not unexpected that the smoking frequency at home was important given that it is the activity that modulates the indoor nicotine concentration. For 3HC only, the employment status of the mother was also an important predictor. The adjusted R^2 is low for the three metabolites, indicating that the models are lacking other factors that are not mentioned in the questionnaire. Factors such as exposure during travelling to or from school, during social activities, the time of exposure or ventilation conditions at home might be important to consider. The need for early development years.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

1. Exposure to secondhand smoke in Maltese school children through urinary biomarkers
2. 95.4% of the children were exposed to nicotine whilst 98.3% were to NNK
3. COT threshold values were used to categorise low to high SHS exposure
4. Higher NNAL/COT ratio in children compared to subgroup defined as smokers
5. Asthmatic children exposed to SHS, had double metabolite levels of non-asthmatics
6. Metabolites influenced mainly by school and house locations and smoking frequency

Figure 1:
School locations in Malta in the different geographical districts

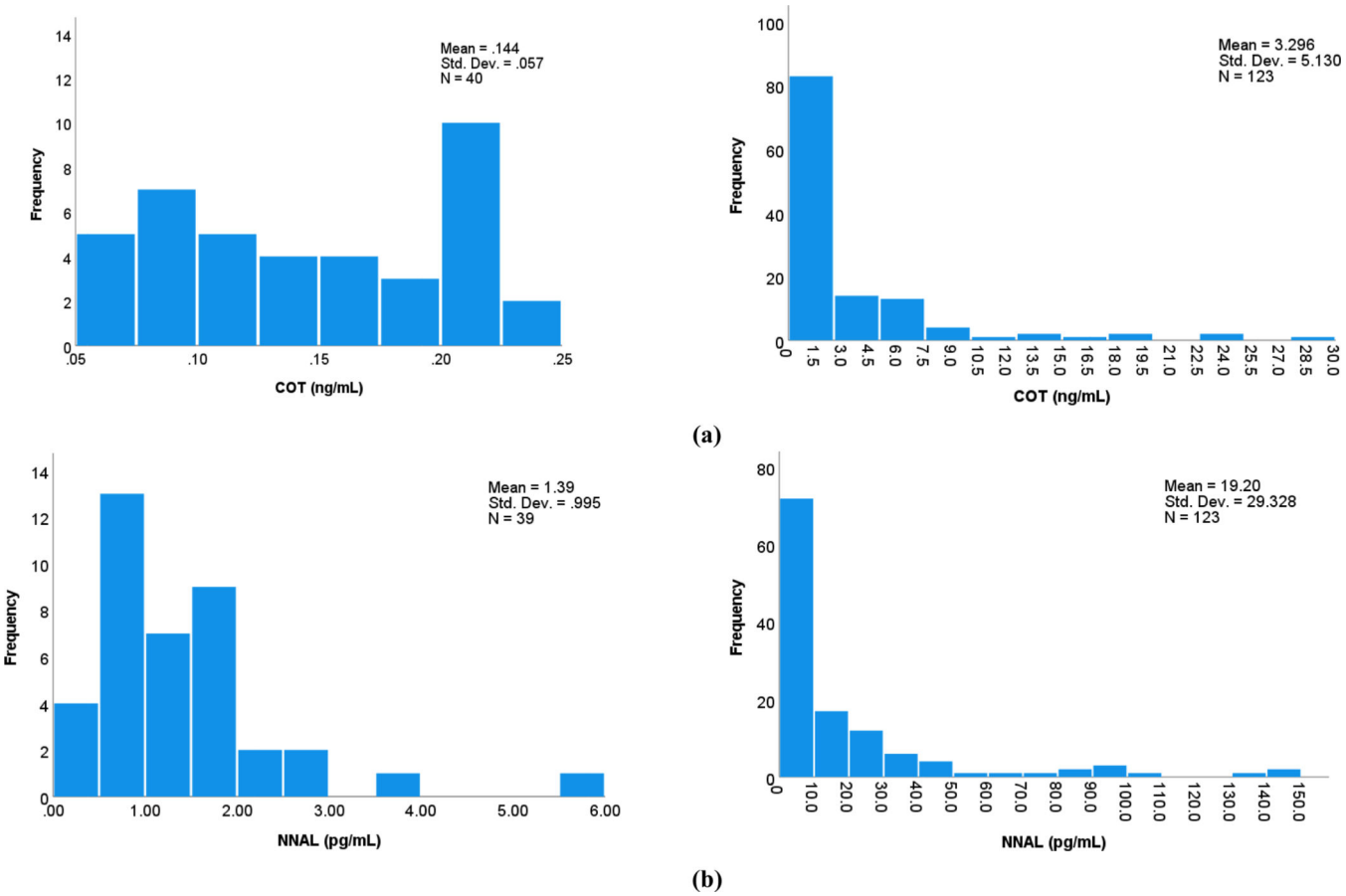
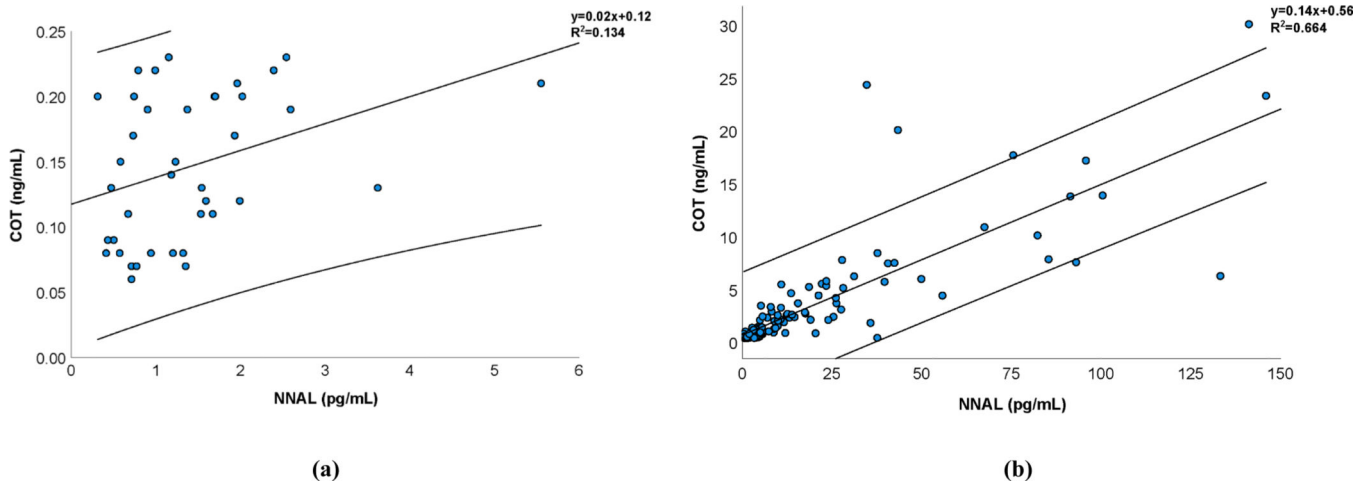


Figure 2:

Number frequency distribution for **low** (left) and **medium** (right) level exposed groups for (a) COT (ng/mL) and (b) NNAL (pg/mL)



(a) **(b)**

Figure 3:

COT vs NNAL for (a) **low** COT exposure and (b) **medium** COT exposure (95% CI lines also shown)

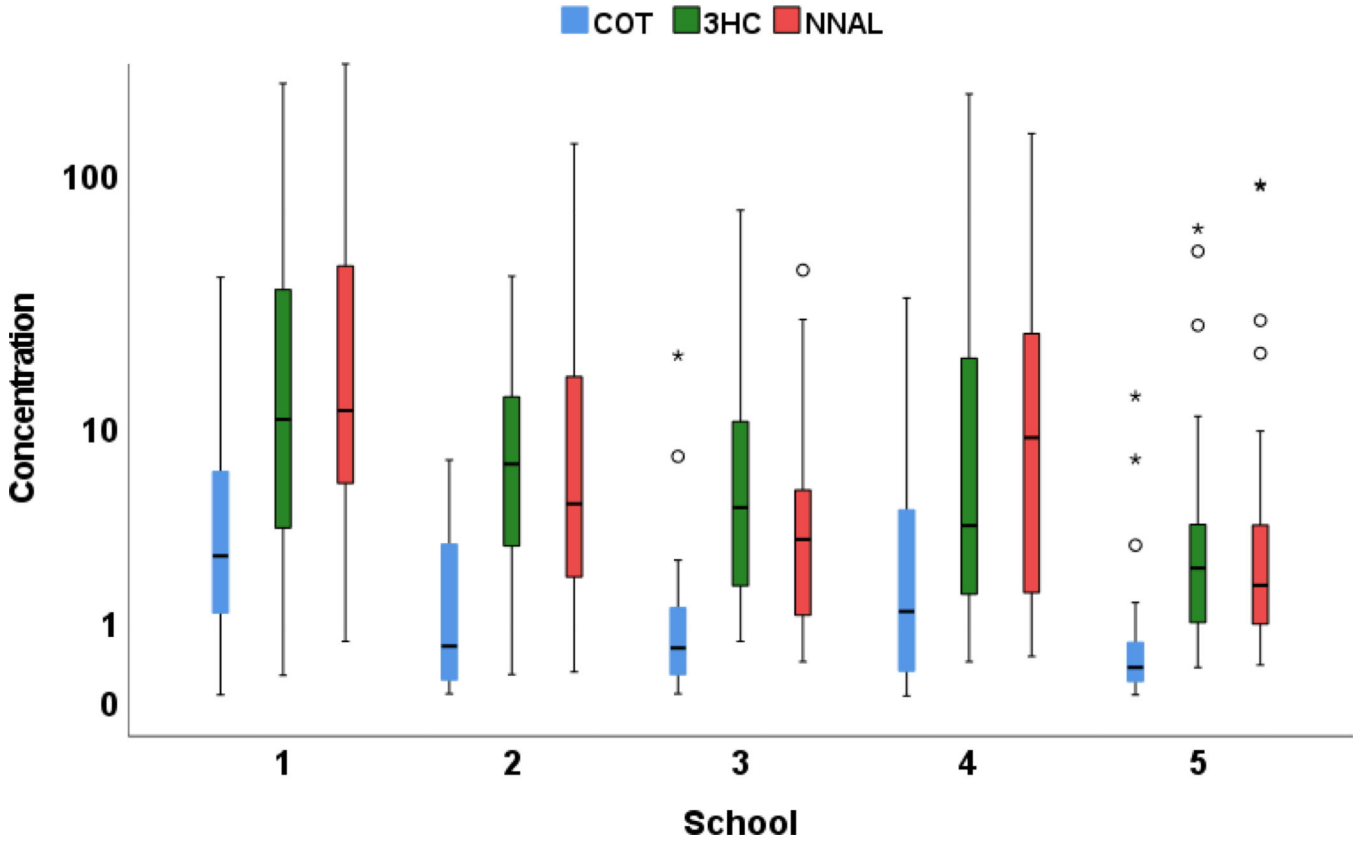


Figure 4:
COT and 3HC concentrations in ng/mL and NNAL in pg/mL obtained in different subgroups by school.

Table 1:

Characteristics of the study group and the corresponding analytical results expressed by the exposure level.

Study	Total	Subjects with COT level (%)			
		(<0.05 ng/mL)	(0.05–0.25 ng/mL)	(0.25–30 ng/mL)	(>30 ng/mL)
		Unexposed Group	Exposed Group		
N (% of sample size)	174 (100)	8 (4.6)	40 (23.0)	123 (70.7)	3 (1.7)
Analytical Data					
COT (ng/mL)	**GM:** 0.9; **AM:** 3.0; **95%CI:** (2.0–3.9); **IQR:** 2.3	NA	0.1; 0.1; (0.1–0.2); 0.1	1.5; 3.4; (2.4–4.3); 3.3	35.3; 35.3; (0–81.3); 8.7
3HC (ng/mL)	**GM:** 4.8; **AM:** 14.1; **95%CI:** (9.7–18.6); **IQR:** 11.6	0.5; 0.7; (0.3–1.2) 0.8	1.1; 1.5; (1.1–1. 9); 1.5	7.4; 16.3; (11.5–21.0); 15.1	106.3; 124.7; (0–1187); -
NNAL (pg/mL)	**GM:** 5.1; **AM: AM:** 18.6; **95%CI:** (11.1–26.1); **IQR:** 13.7	0.7; 1.0; 0.1–1.9 1.8	1.1; 1.4; (1.1–1. 7); 1.0	8.0; 19.5; (14.2–24.8); 20.2	241.4; 318.5; (0–2958); -
NNAL/COT ratio ($\times 10^{-3}$)	**GM:** 5.9; **AM:** 7.9; **95%CI:** (6.2–9.5); **IQR:** 5.8	NA	8.1; 9.7 (7.8–11.5); 8.3	5.3; 7.2; (5.1–9.4); 3.9	6.5; 8.1 (0.9–69.1); -

N = Number of cases; GM = Geometric Mean; AM = Arithmetic Mean; (95% CI) = 95% Confidence Interval of AM; IQR = Interquartile range; NA Not applicable.

Table 2:

Characteristics and analytical results of the exposed group, by asthma diagnosis and smoking status at home.

Analytical Data	Asthmatic		Non-Asthmatic	
	Smoking at Home-No	Smoking at Home-Yes	Smoking at Home-No	Smoking at Home-Yes
N=134 (100%)	24 (17.9%)	15 (11.2%)	73 (54.5%)	22 (16.4%)
COT (ng/mL)	**GM:** 0.6; **AM:** 1.4; **95% CI:** (0.5–2.3); **IQR:** 1.5	3.5; 6.1; (3.3–8.9); 6.0	**GM:** 0.6; **AM:** 2.5; **95% CI:** (0.8–3.3); **IQR:** 1.2	1.5; 4.8; (1.6–8.0); 5.6
3HC (ng/mL)	**GM:** 3.7; **AM:** 11.2; **95% CI:** (0.0–22.3); **IQR:** 4.4	19.2; 30.4; (17.7–43.2); 43.9	**GM:** 3.3; **AM:** 10.7; **95% CI:** (3.5–15.9); **IQR:** 6.0	9.0; 19.6; (11.3–28.0); 34.4
NNAL (pg/mL)	**GM:** 3.5; **AM:** 7.3; **95% CI:** (3.2–11.4); **IQR:** 8.0	21.2; 41.0; (22.0–59.9); 67.3	**GM:** 3.3; **AM:** 9.1; **95% CI:** (4.0–14.3); **IQR:** 7.1	10.9; 34.5; (15.0–54.0); 43.0
NNAL/COT ratio ($\times 10^{-3}$)	**GM:** 5.7; **AM:** 7.1; **95% CI:** (5.4–8.8); **IQR:** 7.0	6.0; 6.7; (4.9–8.5); 5.0	**GM:** 5.5; **AM:** 5.8; **95% CI:** (5.6–7.9); **IQR:** 5.2	7.1; 13.0; (1.4–24.6); 6.3

N = Number of cases; GM = Geometric Mean; AM = Arithmetic Mean; (95% CI) = 95% Confidence Interval of AM; IQR = Interquartile range.

Table 3:

Model parameter estimates for COT, 3HC and NNAL.

Model	Equation	R^2	Adjusted R^2
lnCOT	$1.192+1.136S_1+0.375S_2+0.578S_3+1.020S_4-1.459SF_0-1.288SF_1-0.922HL_0-0.632HL_1$	0.327	0.284
ln3HC	$2.224+1.008S_1+0.443S_2+0.801S_3+1.100S_4-1.862SF_0-1.243SF_1+0.665EM_0+0.232EM_1$	0.293	0.250
lnNNAL	$3.272+0.928S_1+0.405S_2+0.201S_3+0.883S_4-2.051SF_0-1.712SF_1-0.649HL_0-0.485HL_1$	0.375	0.337

Parameters: $S_{1–4}$ = School; $SF_{0–1}$ = Smoking Frequency; $HL_{0–1}$ = House Location; $EM_{0–1}$ = Employment status of mother