ASSessment of Environmental Tobacco Smoke (ETS) Exposure: Urinary Cotinine Concentrations in Children Are Strongly Associated with the House Dust Concentrations of Nicotine at Home

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Abstract
In the present study the possibility of using nicotine in house dust as an index of ETS exposure was evaluated in an environmental investigation of 23 children with asthma. A standardized procedure for house dust sampling of nicotine with a filter holder connected to a vacuum cleaner, for a defined time and area was developed (F-nicotine). Also, house dust sampling was done from the vacuum cleaner bags of the homes (VC-nicotine). There was a larger variation in VC-nicotine (13-655, median 66 µg/g) compared to F-nicotine (15-393 median 156 µg/g). There were statistically significant associations between an inquiry data based ETS exposure index on the one hand, and urinary cotinine concentrations in children (U-cotinine), F-nicotine and VC-nicotine of their homes, on the other. The strong correlation between U-cotinine and F-nicotine (rₛ=0.93; p<0.0001) indicate that the new standardized house dust sampling method should be useful in ETS exposure assessment.

Index Terms
Environmental tobacco smoke, nicotine, cotinine

Introduction
Assessment of environmental tobacco smoke (ETS) exposure in children by the use of questionnaire has a low validity and reliability (Pron et al., 1988; Coultas et al., 1989; Marbury et al., 1993; Kemmeren et al., 1994). The reason for this is, that a large number of factors determine the exposure. Even a detailed questionnaire on ETS exposure gives only a rough picture of the ETS exposure; in fact it is not more precise than one single question on parental smoking habits (Who smokes indoors at home?) only (Willers et al., 2000). Urinary cotinine (U-cotinine; T½~19 h) has been shown to be an excellent biomarker of recent ETS exposure (Willers et al., 1995; Hafroid and Lison, 1998). However, using U-cotinine, Willers et al (2000), found evidence for a differential misclassification of the ETS exposure in asthma studies due to changes in the parental smoking behaviour after symptom debut to reduce ETS exposure of their children. Thus, there is a need for a long-term specific measure of ETS exposure. Air monitoring of nicotine is specific and related to ETS-exposure and U-cotinine, however it reflects short term ETS exposure only (Willers et al., 1995.) (Hein et al., 1991) and (Willers et al., 1993), found high levels of nicotine in house dust from vacuum cleaner bags in smoker’s homes. However, a problem is that the cleaning procedures differ a lot, introducing

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potential bias. Therefore, in the present study a standardized method for dust sampling was evaluated and validated by U-cotinine in children.

METHODS
The environmental investigation was performed in the homes of 23 children, treated in Outpatient Section, Department of Paediatrics, Malmö University Hospital, Sweden during 2 winter months. All participating children, mean age 7.8 (range 1-13), received medical treatment for asthma. The families accepted to join the investigation, including sampling for house dust for the determination of nicotine in their homes and collection of a morning urine sample for the determination of cotinine. None of the children was smoking actively. The children were medically examined at the inclusion visit where also the families were interviewed about smoking habits in their homes. According to the answers on ETS exposure the children were grouped into 5 categories: 0=none of the family members smokes or has smoked since their Childs birth; 1=none smokes indoors or had quit smoking since 6 months 2=only father smokes indoors at home; 3=only mother smokes indoors at home; 4= two family members or more smoke indoors at home.

Sampling of house dust
The house dust sampling was done in two steps:
(1) the parents did vacuum cleaning as usual with their own vacuum cleaner and we collected the whole bag. They were told not to vacuum clean one week before the standardized dust sampling (see below). Two samples (fine and rough fraction) from the vacuum cleaner bag of the home was taken and transferred into two 20 mL plastic tubes, which were analysed for the nicotine content (see below). The mean of these two determinations was used in the tables and calculations.

(2) The house dust was sampled standardized for a defined time (ten minutes) and area (soft and hard surfaces in the living room + place where the child spent most of his time). A filter holder with filter (ALK, Denmark) was connected to a vacuum cleaner (an Oxygen Z 5530; Electrolux, Sweden) with a sucking capacity of 440 W. Sampling was done by the same person (a registered nurse) in all homes.

Determination of nicotine in house dust
The dust content on the vacuum cleaner discs (filters) was analysed for nicotine (F-nicotine) after shaking the untreated dust content with an extraction solution for 60 min. No moisture determination was performed due to the minute amounts of sample. The extraction solution consisted of 400 mL n-hexane with 250 µL n- heptadecane as internal standard. Weighing two g dust, adding 20 mL water, 40 mL extraction solution and ten mL NaOH conc performed the extraction. After separation of the phases the organic phase was transferred to gc-vials for subsequent gaschromatographic analysis. A standard solution was prepared by weighing 0.100 g nicotine into 50 mL water, 100 mL extraction solution ad 25 mL NaOH conc. After shaking the organic nicotine phase was separated into a brown flask and kept below four degrees C. Nine dilutions were prepared from this standard: 0.00125 – 0.00250 – 0.00500 – 0.01000 – 0.01500 – 0.02500 – 0.04000 – 0.05000 – 0.10000 mg/mL nicotine respectively. The calibration curve was practically linear with a linear regression coefficient (r square) typically about 0.99956 for the 9 standard solutions. Running the nine standards followed by ten extracts and the 9 standards performed the gas chromatography. The same procedure was done for analyses of nicotine from the vacuum cleaner bags (VC-nicotine).
Determination of urinary cotinine

Cotinine in urine was determined according to a modified method described by Bernert et al. (1997). One ml of urine was mixed with D3-cotinine as internal standard. The cotinine was extracted with 1 mL of KOH and 7 mL methylenechlorid. The organic phase was evaporated and the samples redissolved in 200 µL of acetonitrile. The samples were analysed using liquid chromatography tandem mass spectrometry (LC-MSMS). The peak area ratios between the analytes and the internal standards were used for quantification. The samples were analysed in duplicates. The detection limit was in the 0.1 ng/mL range and the between day precision as coefficient of variation was 3.6 %.

Statistics

ETS exposure and U-cotinine has a non-normal distribution, therefore non-parametric tests (Mann-Whitney U-test) were performed and medians used for descriptive. Spearman’s rho (rs) was used for calculations of correlations. We used the unadjusted U-cotinine values in the statistical calculations, because some of the children (N=3) were less than 4 yrs old and therefore had very low levels of creatinine, which made these, adjusted estimates uncertain. However, the differences and associations regarding presented parameters became statistically significant also using creatinine-, and density adjusted values (see table 1).

RESULTS

The “non-smoking status” of the children was validated by the U-cotinine levels, which were in the range for non-smokers (<60 µg/L). There was no non-response initially, probably because the investigation is clinical routine besides the nicotine determination. For example, often in clinical practice dust sampling is performed for various allergens. However, there were some missing data on nicotine in vacuum cleaner bags for various reasons e.g. ongoing infections made it difficult to visit the participants at home during the investigation (N=7, 30%); in three of the homes there was not enough dust on the filter (i.e. very low dust content in the home) to allow for a determination of nicotine. Further, there was missing urine cotinine data for 1 subject because he was not at home the day of the house dust sampling.

U-cotinine-, VC-nicotine- and F-nicotine levels are shown in Table 1 and Figure 1.

The differences between homes with (ETS categories 2-4, N=15) and without (0-1, N=8) self-reported current ETS exposure were statistically significant with regard to all studied ETS exposure parameters (U-cotinine, VC-, F-nicotine; Table 1).

Table 1. Medians (ranges within parenthesis) for house dust levels of nicotine and urinary cotinine in children related to parental self-reported current (last 6 months) ETS exposure at home (yes/no). Statistically significant differences are indicated by *=p<0.05 and **p<0.01 (Mann-Whitney U-test).

<table>
<thead>
<tr>
<th>Current ETS exposure</th>
<th>U-cotinine, Unadj (µg/L)</th>
<th>U-cotinine, Densityadj (µg/L)</th>
<th>U-cotinine Creatinineadj (nmol/mmol)</th>
<th>VC nicotine, (µg/g)</th>
<th>F-nicotine (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No (N=8)</td>
<td>1.3 (0.2-15)</td>
<td>1.6 (0.2-12)</td>
<td>1.9 (0.2-26)</td>
<td>31 (18-40)</td>
<td>20 (15-78)</td>
</tr>
<tr>
<td>Yes (N=15)</td>
<td>12** (0.7-55)</td>
<td>13** (0.6-50)</td>
<td>14* (0.3-44)</td>
<td>121* (13-655)</td>
<td>212* (28-393)</td>
</tr>
</tbody>
</table>

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Nicotine in house dust was assessed by a new standardized (time, area) sampling method using a filter disc (ALK, Sweden) connected with a vacuum cleaner with a sucking capacity of 440 W.

ETS exposure category was statistically significantly associated with U-cotinine [(unadjusted: rs=0.62; p<0.002, N=22), (density adj: rs=0.64; p=0.001; N=22), (creatinine adj: rs=0.61; p=0.003; N=22)], VC-nicotine (rs=0.64; p<0.001, N=16), and F-nicotine (rs=0.72; p<0.001, N=13) respectively.

Further, there was an association between VC-nicotine and F-nicotine (rs=0.69; p<0.01, N=13)

There were strong correlations between U-cotinine and VC-nicotine (rs=0.77; p=0.001; N=15) and F-nicotine (Figure 1: rs=0.93; p<0.0001, N=13) respectively.

**DISCUSSION**

The most important findings of the present study is that nicotine concentrations in dust at home were strongly associated with the urinary concentrations of cotinine in children.

To our knowledge, earlier a significant correlation between U-cotinine on the one hand and house dust nicotine on the other has not been presented. Even though nicotine in house dust may contribute to nicotine in air especially with increased indoor ventilation the present association probably is not very important for the ETS dose. U-cotinine is causally associated...
with ETS exposure as earlier was shown by others and us in experimental studies (Skarping et al., 1988; Willers et al., 1995). For example, U-cotinine raised 100-fold after 2-h experimental ETS exposure of 14 children and 7 adults during a bus drive (Willers et al., 1995).

The house dust concentrations of nicotine and urinary concentrations of cotinine in the present study were in good agreement with earlier studies (U-cotinine: Willers et al., 1991; 1992; 1995; 2000, VC-nicotine: Hein et al., 1991; Willers et al., 1993).

Hitherto, the cotinine method has been shown to be superior to other methods for ETS exposure assessment. Further, the present new equipment has made the preparation of the urine samples much easier.

The association between self-reports on ETS exposure (5 categories) and U-cotinine was statistically significant, which earlier has been reported by several others and us. The correlations were not strong which is natural given the rough exposure index.

The results indicate that determination of nicotine in house dust could be complementary to cotinine in urine in ETS exposure assessment. However, we do not know the impact of the cleaning procedures in different homes. Repeated measurements of nicotine in house dust in the same home over a longer period will give more validity information.

A minor disadvantage of the presented method is that the standardized house dust sampling takes many personnel resources. However, it easily could be performed by the patients themselves or in this case their parents. This of course could introduce errors, but the instruction should be easy to follow. There may also be problems achieving enough amounts of dust in homes, which recently has been cleaned.

U-cotinine in children with asthma has earlier been used in parental smoking cessation programs as feedback information to the parents (Willers et al., 1989 and 1991). Probably, also nicotine determination in house dust may have a place in the clinical treatment program of childhood asthma. The sampling for nicotine could be done simultaneously with allergen sampling. It also may be used for Public Health purposes in sample surveys when monitoring the nicotine exposure to the general population.

CONCLUSION AND INTERPRETATION
The strong correlation between U-cotinine and F-nicotine ($r_s=0.93$), indicate that the new standardized house dust sampling method might be useful in ETS exposure assessment.

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