Assessment of Nicotine Dermal Contact and Urinary Cotinine of Tobacco Processing Workers

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Objective: *To assess the dermal contact of nicotine dust, subjective symptoms and urinary cotinine of post curing tobacco workers.*
Material and Method: *Dermal hand wipes of residual nicotine dust samples, morning urine samples and subjective symptoms were collected from 30 workers. The hand-wipe samples and urine samples were analyzed for nicotine and cotinine by a GC/MS, respectively.*
Results: *The average amount of nicotine on the hands of workers was 0.24 microgram/cm^2, while the average urinary cotinine concentration of workers was 3.08 microgram/ml. Moreover, there was a significant correlation between nicotine residue on hands and urinary cotinine excretion at $r = 0.978$, $p < 0.05$. There was also a significant relationship between the occupational related nicotine residue on hands and the number of subjective symptoms reported ($p < 0.05$).*
Conclusion: *The nicotine residue on hands could be used as an indicator of occupational nicotine dust exposure which might affect the health of tobacco workers.*

Keywords: *Nicotine, Cotinine, Tobacco dust, GC/MS*

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Occupational health problems associated with the handling of green tobacco leaves in the field have been reported, especially in America and India[1-5]. It is believed that the etiology of green symptoms was caused by the skin absorption of nicotine of workers who were in prolonged direct contact with nicotine. Such contact may be the causative agent for such sickness[6,7]. Symptoms, including vomiting, giddiness, headache, weakness, and loss of appetite have been found where workers handled dried non-Virginia tobacco leaves[8]. Although these symptoms are not associated with mortality or long-term morbidity, they cause discomfort and a loss of productivity among tobacco workers[1-3].

The processing of drying tobacco leaves actually has various processes[9]. The only process studied here is the post tobacco curing process as its workers have higher dermal contact with nicotine dust. After tobacco is cured, it is moved from the curing barn into a storage area for processing. If whole plants were cut, the leaves would be removed from the tobacco stalks in a process called stripping. For both cut and pulled tobacco, the leaves are then sorted into different grades. They were wrapped by workers, bare handed, into loose piles to be sold. During this process, the workers dermal exposure to nicotine is increased due to greater skin contact. The purpose of this research was to study the workers exposed to

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nicotine through dermal contact, their urinary cotinine and health symptoms, and the relationship between the nicotine concentration on their hands and related urinary cotinine and subjective health symptoms.

Material and Method

Chemicals and reagents

Cotinine (98.0%) was purchased from Sigma-Aldrich, Germany. Nicotine (99.0%) and diphenylamine (98.0%) were purchased from Merck, Germany. All other chemicals were of analytical grades.

Instrumentation

A gas chromatography-mass spectrometer (GC-MS, Trace GC/Polaris Q, U.S.A.) with a DB5-MS Capillary column (30 m x 0.32 mm I.D.) equipped with an ion trap detector was used. The GC condition was as follows: injector, 280°C; detector, 280°C; the column was initially at 50°C for 1 min and then programmed at 15°C min^{-1} to 300°C and held for 5 min. The carrier gas (He) flow rate was 1 ml/min.

A personal air sampler (GILAIR5, Gillian, NJ, USA) was also used.

Study design

A cross-sectional study was carried out to investigate nicotine dust exposure through dermal contact, and the related subjective symptoms and urinary cotinine excretion of workers working in the post tobacco curing process. The research was reviewed and approved in December 2007, by the Ethics Committee on Human Rights Related to Human Experimentation, Mahidol University (Ref. No. MU 2007-247). Field investigation was carried out at the Pak-kwaure community, Muang district, Sukhothai province during the May 2008 post tobacco curing process period.

Subjects

The subjects were 30 tobacco workers working in the post tobacco curing process. After tobacco leaves had been collected and air cured to reduce moisture to less than 12%, the dry tobacco leaves were graded and packed in the post tobacco curing process. There was a lot of dust generated in this process. Subjects were interviewed with questionnaires consisting of general characteristics as well as their subjective symptoms. The hand-wiping method was used to collect nicotine dust on both hands of the workers. Their morning urine was also collected.

Hand-wipe sample collection

The hand-wiping method was used to collect nicotine dust on both hands of post tobacco curing workers. Each sample was collected after 3-4 hours of work before hand cleaning. Nicotine residues on hands were wiped with a cotton wool swab moistened with 10 ml of 100% isopropanol[10], and then each finger of the same hand was wiped with a second cotton wool swab. Both swabs were placed in the same screw-cap vial. Further hand wipe samples were carried out in the same manner and this second group of cotton wool swabs was placed in another screw-cap vial.

Urine sample collection

Midstream morning urine samples were collected from all subjects working in the post tobacco curing process. These samples were immediately stored in ice packs and transferred to a freezer at -20°C until analysed.

Determination of nicotine residue on hands

The method for the analysis of nicotine dust on hands was carried out using a GC/MS[9].

The cotton wool swabs were placed into a screw-cap vial and extracted with 20 ml of hexane-acetone (1:1, v/v) for 1 min with a vortex mixer and the extracted solution was transferred into a 25 ml volumetric flask which was then brought up to a total volume of 25.0 ml with hexane-acetone (1:1, v/v). Exactly 2.0 ml of solution was pipetted and dried. It was reconstituted with 100 microlitres of ethyl acetate and 1 microlitre was injected into the GC/MS. The media blank vials were analysed in the same manner. The nicotine content was converted from microgram/sample to microgram/cm^2 using a surface area of 420 cm^2/hand[10,11].

Desorption efficiency of nicotine from cotton wool swabs

The standard nicotine was prepared at 4.0, 20.0, and 40.0 microgram/ml in ethyl acetate. The cotton wool swabs were spiked with 1.0 ml of these solutions and left to dryness under normal temperature and pressure. The swabs were then placed into screw-cap vials and stored overnight and were then extracted with 20 ml hexane-acetone (1:1, v/v). Extracted solutions were diluted to 25.0 ml with hexane-acetone (1:1, v/v) and then 2.0 ml of the solutions were evaporated under nitrogen blow until dryness. They were reconstituted with 100 microlitres of ethyl acetate and 1 microlitre

was analyzed by GC/MS. The desorption efficiencies were used to correct the amount of nicotine recovered from hand-wipe samples.

Analysis of urinary cotinine in urine samples

The urine samples were analyzed for cotinine concentration following the analytical method by GC/MS[9].

Data analysis

General characteristics were described in terms of number, mean, standard deviation (SD). The relationship between nicotine dust concentrations on hands and urinary cotinine levels was statistically analyzed by the Pearson correlation. The relationship between nicotine dust concentrations on hands and subjective symptoms was performed by the Pearson χ^2-test.

Results

The general characteristics of workers exposed to nicotine dust in the post tobacco curing process are shown in Table 1. There were 23 male and 7 female subjects. Their average age was 51.7 years old and two of them were smokers.

Table 1. The general characteristics of workers in the post tobacco curing process

Characteristics	Number of subjects	%
Sex		
Male	23	76.7
Female	7	23.3
Age (years)		
< 40	6	20.0
40-49	5	16.7
50-59	13	43.3
60 +	6	20.0
Mean $\pm$ SD	(51.70 $\pm$ 12.94)	
Working experience (years)		
< 10	3	10.0
10-19	9	30.0
20-29	8	26.6
30-39	5	16.7
40 +	5	16.7
Mean $\pm$ SD	(29.40 $\pm$ 11.44)	
Cigarette smoking		
Yes	2	6.7
No	28	93.3
Alcohol consumption		
Yes	15	50.0
No	15	50.0

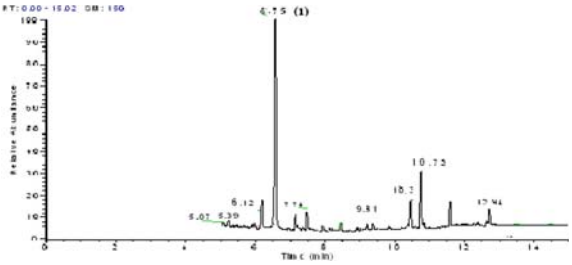

Fig. 1 Chromatogram of a hand-wipe sample of a worker obtained by a GC/MS using the SIM technique. The peak identified is nicotine (1)

Analysis of nicotine residue on hands

The chromatogram in Fig. 1 shows the peak of nicotine from a hand-wipe sample. The nicotine had a retention time of 6.75 min. The analysis time was completed within 14 min. The ions at m/z 84 and 133 were monitored for nicotine.

Desorption efficiency of nicotine from cotton wool swabs

The desorption efficiencies of nicotine at 5, 25, and 50 mg from cotton wool swabs were 77.8, 81.9 and 86.1%, respectively. The desorption efficiencies were used for correction of the amount of nicotine detected on the hands of workers.

Concentration of nicotine residue on hands

The concentrations of nicotine from hand-wipe samples are shown in Table 2. Thirty workers had dermal contact with nicotine concentration ranging from 0.07 to 0.37 microgram/cm^2. The average nicotine dust concentration on the left hands of 20 workers was 0.21 $\pm$ 0.07 microgram/cm^2, while the average nicotine dust concentration for the right hands of 16 workers was 0.19 $\pm$ 0.07 microgram/cm^2. There is no guideline or recommendations by NIOSH or other regulatory agencies for skin contact with nicotine[12,13].

Symptoms of workers in the post tobacco curing process

The most reported symptoms of workers were weakness, increased perspiration, dizziness, nausea and headache (Table 3).

Urinary cotinine concentration of the post tobacco curing process.

The median urinary cotinine concentrations of the 28 non-smoking subjects in the post tobacco curing period were 3.03 microgram/ml ranging from 0.20

Table 2. Nicotine concentrations for the left and right hands of 30 tobacco workers in the post tobacco curing process

	Nicotine conc. (microgram/cm^2)	n	Mean ± SD (microgram/cm^2)	Range (microgram/cm^2)
Left hand	<0.1	2	0.08 ± 0.02	0.07-0.09
	0.1-0.3	20	0.21 ± 0.07	0.11-0.30
	>0.3	8	0.33 ± 0.02	0.31-0.36
Right hand	<0.1	1	0.09 ± 0.00	0.09-0.09
	0.1-0.3	16	0.19 ± 0.07	0.11-0.29
	>0.3	13	0.34 ± 0.02	0.31-0.37

Remark: Surface area of each hand approximately 420 cm^2

Table 3. The frequent symptoms reported by workers in the post curing process

Symptoms	Number of workers reported (%)
Weakness	19 (63.3)
Increased perspiration	19 (63.3)
Dizziness	18 (60.0)
Nausea	15 (50.0)
Headache	13 (43.3)
Vomiting	4 (13.3)
Pallor	4 (13.3)
Shortness of breath	4 (13.3)
Fluctuation in heart rate	3 (10.0)
Fluctuation in blood pressure	3 (10.0)

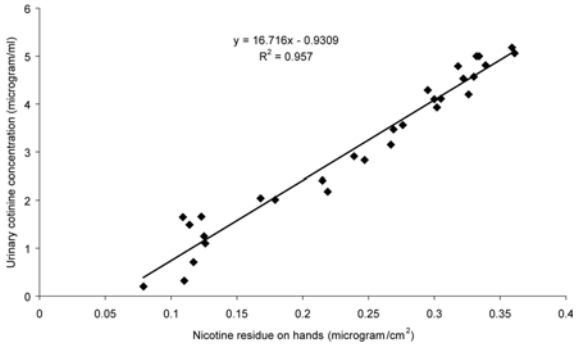

Fig. 2 Scatter diagram between nicotine residue on hands (microgram/cm^2) and urinary cotinine concentration (microgram/ml) of workers in the post tobacco curing process

Table 4. Range and median of urinary cotinine concentrations of workers in the post tobacco curing process

	Range and median of urinary cotinine concentration (microgram/ml)		
	No. of workers	Median	Range
Non-smoking	28	3.03	0.20-5.18
Smoking	2	-	3.56-4.57

to 5.18 microgram/ml (Table 4). The 2 smoking subjects had urinary cotinine concentrations at 3.56 and 4.57 microgram/ml.

The relationship between nicotine dust on hands and urinary cotinine levels of workers in the post tobacco curing process

The correlation coefficient of nicotine dust on hands (μg/cm^2) and urinary cotinine concentration (microgram/ml) was 0.978 ($p < 0.05$). The equation was $Y = 16.716\ X - 0.9309$ and the coefficient of determination (R^2) was 0.957 indicating that the nicotine residue on hands had a linear correlation with urinary cotinine concentrations. The scatter diagram showing nicotine dust residue on hands and urinary cotinine concentrations of workers is shown in Fig. 2.

The relationship between nicotine dust on hands and the subjective symptoms of workers

The relationship between nicotine dust on hands and the subjective symptoms of the 30 workers in the post tobacco curing process was determined and calculated by the Pearson χ^2-test. It was found that when the concentration of nicotine dust on the workers hands was less than the mean value of 0.24 microgram/cm^2 workers developed less symptoms than those workers with nicotine dust on their hands of an amount higher than the mean value of 0.24 microgram/cm^2 (Fig. 3). The factor that had a significant relationship with the number of symptoms was a concentration of

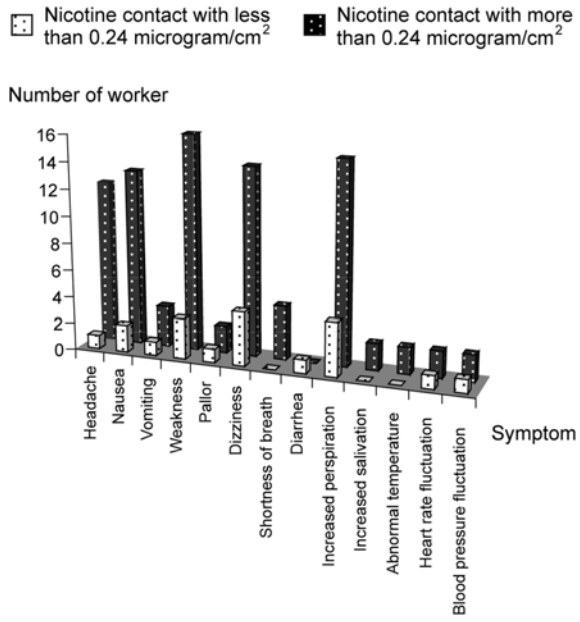

Fig. 3. Number of workers having symptoms at different levels of dermal contact with nicotine.

nicotine dust on hands with an association of 18.22 ($p < 0.05$), indicating that the concentration of nicotine dust on hands had an association with the number of symptoms reported by workers.

Discussion

The concentrations of nicotine dust on hands among workers in the post tobacco curing process

The average nicotine dust on the hands of 30 workers in the dry tobacco leaf preparation area was 0.24 microgram/cm^2 ranging from 0.07 to 0.37 microgram/cm^2. There is no guideline or recommendations by NIOSH or other regulatory agencies in regards to skin exposure[13]. The nicotine dust concentrations on the hands of workers in this study were lower than those of previous studies, because these concentrations of nicotine were measured from nicotine dust on hands, while in the previous study the concentrations of nicotine were measured from nicotine dew which was nicotine dust on the hands of harvesters before hand washing. The nicotine concentration for the pre-wash hand-wipe samples was 10 microgram/cm^2 while the post-wash hand-wipe sample was 0.38 microgram/cm^2[11].

However, tobacco workers can be subjected to dermal absorption of tobacco dust. It is possible that moisture (sweating in summer) may promote the absorption of nicotine from this dust in a similar way as moisture has promoted green symptoms among the harvesters[6].

The relationship between nicotine dust residue on hands and urinary cotinine concentrations of workers

The correlation between nicotine residue on hands (microgram/cm^2) and urinary cotinine concentrations (μg/ml) among workers was = 0.978, $p < 0.05$. This result indicated that urinary cotinine concentration had a high correlation with the nicotine dust residue on hands which was absorbed through dermal contact.

The relationship between nicotine dust residue on hands and the subjective symptoms of workers

The relationship between the nicotine dust residue on hands and the subjective symptoms reported was determined in 30 workers in the post tobacco curing process. There was a significant correlation between the level of nicotine dust residue on hands (microgram/cm^2) and the number of symptoms reported among workers at $p < 0.025$. These results indicated that the concentration of nicotine had a relationship with the subjective symptoms reported among workers during work shifts. The nicotine dust exposure from this dermal route may promote the absorption of nicotine from dust more than inhalation because of the moisture from sweat in summer.

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การประเมินการสัมผัสนิโคตินทางผิวหนัง และโคตินินในปัสสาวะของคนงานเตรียมใบยาสูบ

พรพิมล กองทิพย์, อมรชัย ไตรคุณากรวงศ์, สุทธินันท์ ฉันท์ธนกุล, วิทยา อยู่สุข, ปรีชา ลอเสรีวานิช, เพียงจันทร์ โรจนวิภาต

วัตถุประสงค์*: เพื่อประเมินการรับสัมผัสฝุ่นนิโคตินทางผิวหนัง อาการ และนิโคตินในปัสสาวะในคนงานเตรียมใบยาสูบแห้ง*

วัสดุและวิธีการ*: เก็บตัวอย่างการรับสัมผัสฝุ่นนิโคตินที่มือ ปัสสาวะตอนเช้า อาการของคนงาน 30 คน นำตัวอย่างที่เก็บจากมือ และปัสสาวะไปวิเคราะห์หานิโคตินและโคตินินโดยใช้แก๊สโครมาโตกราฟี/แมสสเปคโตรมิเตอร์ (GC/MS) ตามลำดับ*

ผลการศึกษา*: ค่าเฉลี่ยนิโคตินที่ติดที่มือคนงานเป็น 0.24 ไมโครกรัมต่อตารางเซนติเมตร ในขณะที่ความเข้มข้นเฉลี่ยโคตินินในปัสสาวะคนงานเป็น 3.08 ไมโครกรัม/มิลลิลิตร และพบความสัมพันธ์สูงอย่างมีนัยสำคัญทางสถิติระหว่างความเข้มข้นนิโคตินที่มือและโคตินินในปัสสาวะมีค่าสัมประสิทธิ์สหสัมพันธ์ (r) เท่ากับ 0.978 ($p < 0.05$) พบความสัมพันธ์อย่างมีนัยสำคัญทางสถิติระหว่างนิโคตินที่มือคนงานจากการทำงานและจำนวนอาการที่รายงาน ($p < 0.05$)*

สรุป*: นิโคตินที่มือคนงานสามารถใช้เป็นตัวบ่งชี้การรับสัมผัสฝุ่นนิโคตินจากการทำงานกับใบยาสูบแห้งและอาจจะมีผลทำให้คนงานเกิดอาการทางสุขภาพ*