Assessment of Nicotine Inhalation Exposure and Urinary Cotinine of Tobacco Processing Workers

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Objective: *To study the composition of tobacco dust, atmospheric nicotine concentration, urinary cotinine excretion and the subjective symptoms of workers in dry tobacco leaf preparation.*
Material and Method: *The tobacco dust in air of the breathing zone of workers and the urine samples of these workers and a comparison group were collected and analyzed by GC/MS. The accuracy, precision and detection limit of the methods were determined.*
Results: *The tobacco dust contained nicotine and atrazine (a herbicide). The average atmospheric nicotine was 0.105 mg/m^3 and urinary cotinine concentrations of post tobacco curing process workers was 3.084 microgram/ml. Moreover, there was a significant correlation between the atmospheric nicotine dust and urinary cotinine excretion ($r = 0.987$, $p < 0.05$). The health symptoms of headache, nausea, weakness, dizziness, and increased perspiration reported among workers had a significant relationship with the job characteristics of the post tobacco curing process workers, with a p-value < 0.05.*
Conclusion: *Nicotine dust contained a herbicide called atrazine. Nicotine concentrations were highest in the post tobacco curing process where workers reported a lot of adverse symptoms. Urinary cotinine can be used as a biomarker of tobacco dusts' exposure in dry tobacco leave preparation areas.*

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

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Green tobacco sickness has been investigated in groups of tobacco harvesters for a long time[1-5]. It is believed that the illness is caused by dermal absorption of nicotine after long term contact with nicotine[6,7]. The characteristic health symptoms of vomiting, giddiness, headache, tiredness, and loss of appetite, occur on the work days after exposure to heavy dust[8].

The processing of dry tobacco leaves involves various processes; (a) tobacco leaves are threshed from stems and (b) air cured to limit their moisture. After that, (c) leaves are graded and grasped, and the products were sent to the factory. Tobacco dust is generated during these processes. Furthermore, tobacco dust from cleaning during the day is also distributed into the workplace environment. People who work in tobacco processing continuously inhale and dermally absorb tobacco dust including nicotine and other harmful constituents of tobacco. These risk factors may affect the health of workers. The purpose of this research was to study the composition of tobacco dust, atmospheric nicotine concentration, urinary cotinine excretion, and the symptoms of workers and the relationship between atmospheric nicotine concentration, urinary cotinine and the workers symptoms.

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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. The other chemicals were of analytical grades.

Instrumentation

A gas chromatography-mass spectrometer (GC-MS, Trace GC/Polaris Q, USA) 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, 300°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.

Personal air sampler (GILAIR5, Gillian, NJ, USA).

Study design

A cross-sectional study was carried out to investigate nicotine dust exposure symptoms and the urinary cotinine excretion of workers in dry tobacco leaf preparation. The research was reviewed and approved by the Ethics Committee on Human Rights Related to Human Experimentation, Mahidol University. Field investigation was carried out in the Pak-kwaure community, Muang district, Sukhothai province during the April 2008 tobacco curing process and the May 2008 post tobacco curing process.

Subjects

There were 30 workers in each of the two groups: a) the tobacco curing process group, and b) the post tobacco curing process group. The inclusion criteria of workers are male or female workers working in dry tobacco leave preparation process in the community. The two groups comprised the same subjects except for three workers. The three smokers in the tobacco curing process group did not participate in the post tobacco curing process. The comparison group was 30 people from the same community who did not work in dry tobacco leaf preparation. The subjects were interviewed with questionnaires consisting of general characteristics, working factors and work related health symptoms. Samples of air from the workers breathing zone, urine samples, and dermal contacts with nicotine dust were collected, while the results of dermal contact with nicotine were presented elsewhere.

Personal air sample collection

Air samples in the breathing zone of workers in the two tobacco processing areas were collected using 3.7 mm cassettes containing PVC filters connected with personal air sampling pumps at a flow rate of 1.0 L/min for three hours. The cassette filter holders containing PVC filters that were not drawn the air through served as field blanks.

Urine sample collection

Midstream urine samples were collected from all subjects during the periods of the tobacco curing and post tobacco curing processes in the morning of the day of their interview and immediately stored in ice packs and transferred to a freezer at -20°C until analysis.

Determination of nicotine in air

Calibration curve of nicotine determination

A standard nicotine solution was prepared at 1.0, 5.0, 25.0, 50.0 with 100 microgram/ml in ethyl acetate and 1 microlitre was injected into the GC/MS for three replications. The detection limit of the method was determined following the National Institute for Occupational Safety and Health (NIOSH) method[9].

Accuracy and precision of the nicotine determination method

The accuracy and precision of the method was determined by analysis of known concentrations of nicotine: 5.0, 25.0, and 50 microgram/ml in ethyl acetate for three replications and for three days of analysis. Exactly 1 microlitre was injected into the GC/MS.

Analysis of atmospheric nicotine concentration

The PVC filters were placed in 10-ml test tubes and rinsed with 5 ml of hexane-acetone (1:1, v/v), and mixed for 1 min with a vortex mixer and then centrifuged. Exactly 2 ml of the supernatants were pipetted and evaporated under nitrogen blow until dryness, and then reconstituted with 100 microlitre of ethyl acetate. One microlitre of the sample was injected into the GC/MS.

Desorption efficiency of nicotine from PVC filter

Three nicotine standard solutions of 20.0, 40.0, and 100.0 microgram/ml were prepared. One hundred microlitre of each of these solutions were spiked on a PVC filter and left to dry under normal temperature and pressure overnight. Each PVC filter

was rinsed with 5 ml of hexane-acetone (1:1, v/v), and then 2 ml were pipetted and put into a test tube and evaporated to dryness under nitrogen blow. It was reconstituted with 100 microlitre of ethyl acetate and 1 microlitre was then analyzed by a GC/MS. A desorption efficiency was used to correct the amount of nicotine recovered from each PVC filter.

Analysis of urinary cotinine in urine samples

Two ml of normal urine containing standard cotinine ranging from 1 to 100 microgram/ml, urine samples, quality control samples, and blank urine samples were placed into 10-ml glass stopper test tubes, adjusted to pH 9 using 10.0% sodium carbonate and 10.0 microlitre of diphenylamine (10 mg/l of methanol) as internal standard were added. The solution was extracted with 5.0 ml of dichloromethane, centrifuged, and the lower layer of dichloromethane was kept to evaporate under nitrogen blow until dryness. It was reconstituted with 200 microlitre of toluene and 1 microlitre of solution was analyzed with a GC/MS. The detection limit of the method was performed following the NIOSH method[9].

Accuracy and precision of the cotinine determination method

The accuracy and precision of the method was evaluated by analyzing the three different cotinine concentrations (5.0, 25.0, and 50.0 microgram/ml, respectively) for five replications. The analysis was carried out as described above.

Data analysis

The general characteristics of all variables were described in terms of percentage, mean, standard deviation (SD). The relationship between atmospheric nicotine dust and urinary cotinine concentrations was done using the Pearson correlation. The relationship between job characteristics and subjective symptoms was performed by the χ2-McNemar test.

Results

The general characteristics of workers exposed to nicotine dust in dry tobacco leaf preparation and the comparison group are tabulated below (Table 1). The workers in the tobacco curing process group and the post tobacco curing process were almost the same,

Table 1. The general characteristics of workers exposed to nicotine dust in a dry tobacco leaf preparation area

Characteristics	Number of subjects		
	Tobacco curing process	Post tobacco curing process	Comparison group
Sex			
Male	23	23	22
Female	7	7	8
Age (years)			
< 40	4	6	13
40-49	6	5	6
50-59	14	13	10
60 +	6	6	1
Mean $\pm$ SD	(52.87 $\pm$ 11.99)	(51.70 $\pm$ 12.94)	(42.07 $\pm$ 11.72)
Working experience/(years)			
< 10	2	3	-
10-19	8	9	-
20-29	10	8	-
30-39	6	5	-
40 +	4	5	-
Mean $\pm$ SD	(29.90 $\pm$ 11.26)	(29.40 $\pm$ 11.44)	
Cigarette smoking			
Yes	5	2	4
No	25	28	26
Alcohol consumption			
Yes	13	15	13
No	17	15	17

except that the three smokers from the tobacco curing process group were not in the post tobacco curing process group. There were no significant differences between the two groups of subjects in regards to sex, age, and years of working experience with tobacco. None of the workers used any protective devices.

Determination of atmospheric nicotine concentration
Calibration curve of nicotine determination

The standard curve of nicotine was linear over the concentration range of 0.87-100.07 μg/ml. The standard curves of relative peak areas (y) against nicotine concentrations (x) was y = 853137x + 1.71191E + 06 at the correlation coefficient of 0.9998. The detection limit of the method for nicotine determination was 2.02 ng/ml.

Accuracy and precision of the nicotine determination

The percent recoveries of the method for analysis of nicotine at the concentrations of 5.0, 25.0, and 50.0 microgram/ml. ranged from 94.30% to 101.19% for nicotine. The between-day assay coefficients of variation were in the range of 0.36% to 3.95%.

Desorption efficiency of nicotine from a PVC filter

The desorption efficiencies of nicotine (n = 3) at 5, 25 and 50 micrograms from a PVC filter were 89.60, 90.20 and 90.48 %, respectively.

Composition of tobacco dust

The results of workplace air composition are presented in Fig. 1. The chromatogram showed the peaks of nicotine and atrazine at retention times of 6.79 and 12.79 min. Although there were some interference peaks of column breeding at retention times of 11.24 and 12.05 min, the retention time of septum breeding was 10.55 min. There was some natural sesquiterpenoid product from plants at a retention time of 7.12 min on the chromatogram.

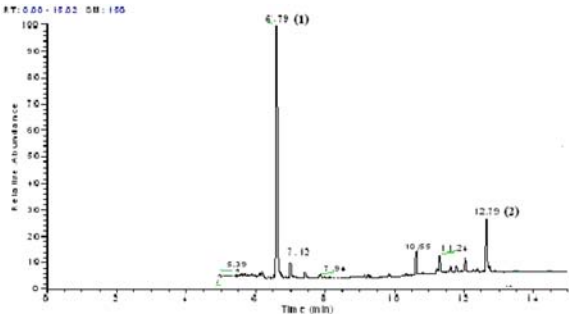

Fig. 1 Chromatograms of air composition from a workplace obtained by GC/MS. The peaks were identified as nicotine (1) and atrazine (2)

Atmospheric nicotine concentration

The results of atmospheric nicotine concentration from personal air sampling are shown in Table 2. The thirty workers were exposed to nicotine concentrations in the range of 0.047-0.154 mg/m^3, which was lower than the American Conference of Governmental Industrial Hygienists (ACGIH) TLV-TWA recommended threshold limit of 0.5mg/m^3 [10].

Analysis of urinary cotinine in urine samples
Calibration curve for urinary cotinine determination

The standard curve of nicotine was linear over the concentration range of 0.0085-5.084 microgram/ml with the equation of y = 0.913x + 0.0294, when y = relative peak area and x = nicotine concentration. The correlation coefficient of the calibration was 0.9992 and n = 3. The detection limit of urinary nicotine determination was 1.19 ng/ml.

Accuracy and precision of the cotinine determination method

The percent recoveries of the urinary cotinine analysis method ranged from 97.11% to 102.21% at

Table 2. Atmospheric nicotine concentrations of 30 air samples in the post tobacco curing process

Nicotine conc. (mg/m^3)	n	Mean ± SD (mg/m^3)	Range (mg/m^3)
< 0.05	2	0.048 ± 0.001	0.047-0.048
0.05-0.10	11	0.073 ± 0.020	0.051-0.100
0.101-0.15	16	0.132 ± 0.013	0.109-0.149
> 0.15	1	0.154 ± 0.000	0.154-0.154

Table 3. The between-day assay for the determination of accuracy and precision of urinary cotinine analysis

Known conc. (μg/ml)	Analyzed conc. Mean ± SD	% CV Between-day (n = 3)	Average recovery (%)
0.5	0.49 ± 0.02	3.18	97.11
1.0	1.02 ± 0.02	2.17	102.21
2.5	2.47 ± 0.04	1.47	98.61

cotinine concentrations of 0.5-2.5 microgram/ml. The between-day assay (n = 3) coefficient of variation was in the range of 1.47% to 3.18%.

Analysis of urinary cotinine in urine samples

The chromatogram shows the peaks of nicotine, diphenylamine and cotinine at retention times of 6.83, 9.20, and 9.82, respectively (Fig. 2). Total GC/MS run time per sample was approximately 14 min. The ions at m/z 98, 176, and 118 were monitored for cotinine and the ions at m/z 169 and 168 were monitored for diphenylamine.

Urinary cotinine concentrations of workers and the comparison group

The urinary cotinine concentrations in the workers and the comparison group were not detected in the tobacco curing process.

Regarding the post tobacco curing process, the median urinary cotinine concentration of the 28 non-smoking workers was 3.03 micrograms/ml, ranging from 0.20 to 5.18 micrograms/ml. The urinary cotinine concentrations for the non-smoking comparison group were not detected. The post tobacco curing workers had the highest urinary cotinine excretion levels.

The relationship between atmospheric nicotine concentration and urinary cotinine concentrations of workers in the post tobacco curing process

The correlation coefficient between atmospheric nicotine dust concentrations (mg/m^3) and urinary cotinine excretion concentrations (microgram/ml) was 0.987 ($p < 0.05$). The scatter diagram, Fig. 3, shows atmospheric nicotine concentrations and urinary cotinine concentrations. The equation was y = 42.519x-1.392. The coefficient of determination (R^2) was 0.975, indicating that atmospheric nicotine concentration had a linear correlation with urinary cotinine excretion.

The relationship between job characteristics and the subjective symptoms of workers

Fig. 4 shows the subjective symptoms in each job category experienced by the workers.

The symptoms of headache, nausea, weakness, dizziness, and increased perspiration occurrence among workers had a significant relationship with the job characteristics of the post tobacco curing process, with p-value < 0.05, indicating that job characteristics had an association with some symptoms occurring in workers while working.

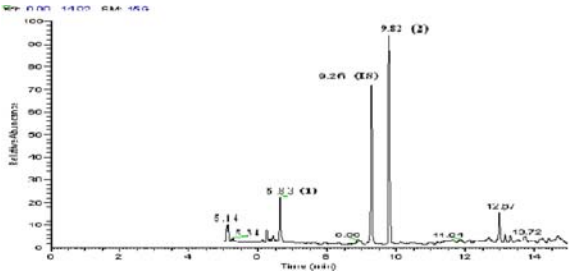

Fig. 2 Chromatogram of a urine sample from an exposed worker obtained by GC/MS using the SIM technique. The peaks were identified as nicotine (1), diphenylamine (IS), and cotinine (2)

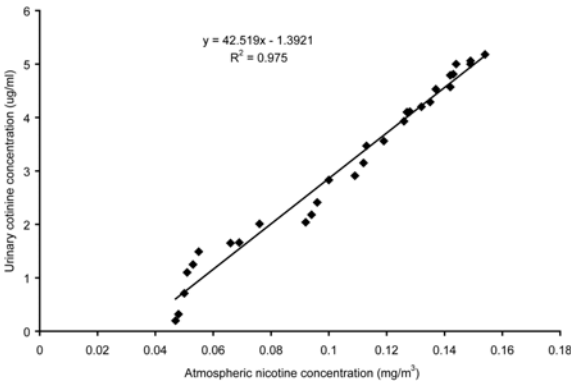

Fig. 3 Scatter diagram between atmospheric nicotine concentrations (mg/m^3) and urinary cotinine concentrations (microgram/ml) of workers in the post tobacco curing process

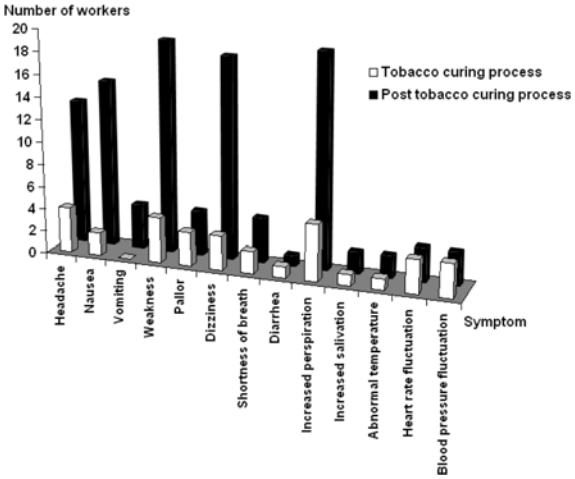

Fig. 4 Number of workers having subjective symptoms in each job category

Table 4. Range and median of urinary cotinine concentrations of workers and the comparison group classified by tobacco curing and post-tobacco curing processes

Process	Smoking status	Urinary cotinine concentration (microgram/ml)					
		Workers			Comparison group		
		n	Median	Range	n	Median	Range
Tobacco curing	Non smoking	25	-	0.00-0.00	26	-	0.00-0.00
	Smoking	5	1.67	1.15-2.10	4	0.84	0.69-1.27
Post tobacco curing	Non smoking	28	3.03	0.20-5.18	26	-	0.00-0.00
	Smoking	2	3.56, 4.57	3.56-4.57	4	1.04	0.54-2.01

Discussion

All of the workers (100%) were exposed to a nicotine level of less than 0.5 mg/m^3, which was in compliance with the ACGIH TLV-TWA recommended threshold limit. The environmental nicotine concentrations in another tobacco processing area was 1.18 mg/m^3 during working hours, while during the non-working hours the value was 0.25 mg/m^3[8]. The nicotine exposure among workers in this study was less than that of the previous study because the dry tobacco leaf preparation area had good natural ventilation as well as some electric fans. Therefore, all workers were exposed to nicotine concentrations below the ACGIH TLV-TWA recommended limit. A significant correlation between atmospheric nicotine concentrations (mg/m^3) and urinary cotinine concentrations (microgram/ml) among workers was found (r = 0.987), indicating that urinary cotinine can be used as a biomarker of tobacco dusts' exposure in dry tobacco leave preparation areas. The cotinine having long half-life in the body is commonly used but it is affected by inter-individual differences in nicotine metabolism[11]. The symptoms of these tobacco processing workers resembled those of the workers in tobacco cultivation. The subjective symptoms such as headache, nausea, weakness, dizziness, and increased perspiration of workers had a significant relationship with the job characteristics of the tobacco post-curing process at p-value < 0.05, indicating that the job characteristics could cause some of these symptoms.

Activities during work affected the level of exposure to nicotine. Workers in the tobacco curing process cut and pulled out tobacco leaves and hung them in well-ventilated barns allowing them to dry, while workers in the post tobacco curing process removed tobacco leaves sorting them into different grades. They worked for the whole day and everyday. They were exposed to nicotine dust through both inhalation and dermal contact for prolonged periods of time and developed some symptoms.

Atrazine, a herbicide remaining in tobacco dust, may be the cause of a variety of health effects[12,13] such as shortness of breath, nausea, vomiting, diarrhea, and increased perspiration. The criteria of OSHA PEL and ACGIH TLV for atrazine are the same for an 8-hour TWA concentration of 5 mg/m^3[14]. As a result, some symptoms found in workers are probably caused by atrazine remaining on tobacco leaves in addition to residual nicotine dust.

Conclusion

Nicotine dust contained a herbicide called atrazine. Nicotine concentrations were highest in the post tobacco curing process where workers reported a lot of adverse symptoms. Urinary cotinine can be used as a biomarker of tobacco dusts' exposure in dry tobacco leave preparation areas.

Acknowledgments

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การประเมินการสัมผัสนิโคตินจากการหายใจ และโคตินีนในปัสสาวะของคนงานเตรียมใบยาสูบ

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

วัตถุประสงค์*: เพื่อศึกษาองค์ประกอบของฝุ่นใบยาสูบ ความเข้มข้นของนิโคตินในบรรยากาศ ความเข้มข้นของโคตินินที่ขับถ่ายทางปัสสาวะและอาการของคนงานในการเตรียมใบยาสูบแห้ง*

วัสดุและวิธีการ*: เก็บตัวอย่างฝุ่นยาสูบในอากาศและปัสสาวะของคนงานบ่มใบยา คัดเกรดใบยาและกลุ่มเปรียบเทียบ และวิเคราะห์โดยใช้แก๊สโครมาโตกราฟี/แมสสเปคโตรมิเตอร์ (GC/MS) ตรวจสอบความถูกต้อง ความแม่นยำ และความไวของวิธีวิเคราะห์ด้วย*

ผลการศึกษา*: ฝุ่นยาสูบประกอบด้วยนิโคตินและอะทราซีน (สารปราบศัตรูพืช) ค่าเฉลี่ยความเข้มข้นนิโคตินในบรรยากาศ 0.105 มิลลิกรัมต่อลูกบาศก์เมตร และโคตินินในปัสสาวะของคนงาน 3.084 ไมโครกรัมต่อมิลลิลิตร ในคนงานคัดเกรดใบยา และพบความสัมพันธ์สูงอย่างมีนัยสำคัญทางสถิติระหว่างความเข้มข้นนิโคตินในบรรยากาศและโคตินินในปัสสาวะมีค่าสัมประสิทธิ์สหสัมพันธ์ (r) เท่ากับ 0.978 ($p < 0.05$) อาการที่คนงานรายงานคือ ปวดศีรษะ คลื่นเหียน อ่อนเพลีย วิงเวียน และเหงื่อออกมาก มีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติกับลักษณะงานของคนงานคัดเกรดใบยา ที่ค่า $p < 0.05$*

สรุป*: ฝุ่นนิโคตินมีสารปราบศัตรูพืชคืออะทราซีน พบความเข้มข้นของโคตินินในปัสาวะสูงสุดในกลุ่มคนงานคัดเกรดใบยาซึ่งคนงานรายงานอาการทางสุขภาพหลายอย่าง*