Exposure to Trinitrotoluene and Health Effects among Workers in an Artillery and Ammunition Plant

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Objective: *To determine urinary trinitrotoluene (TNT), 2-amino-4, 6-dinitrotoluene (2ADNT) and 4-amino-2, 6-dinitrotoluene (4ADNT) and health effects upon workers in an ammunition plant.*
Material and Method: *The urine samples from forty munition workers and forty office workers were monitored for TNT and its metabolites by gas chromatography-mass spectrometry. The workers were interviewed with a questionnaire.*
Results: *The median levels of TNT and its metabolites were 112.84 and ranged from non-detectable (ND) to 1,833.81 mg/L. Median levels for 2ADNT were 11.66, ranging from ND to 360.89 mg/L. Median levels for 4ADNT were 19.95 and ranged from ND to 314.28 mg/L. There were significant correlations between TNT and 2ADNT in urine ($r = 0.700$, p-value < 0.001), as well as for TNT and 4ADNT ($r = 0.783$, p-value < 0.001). Exposed workers reported eye, nose and throat irritations, weakness and headaches with considerably higher frequency than non-exposed workers.*
Conclusion: *TNT levels in urine were strongly associated with 4ADNT and 2ADNT levels. Workers exposed to TNT complained of nose, throat and eye irritation, along with overall weakness and headaches.*

Keywords: *2,4,6-Trinitrotoluene (TNT), 2-amino-4,6-dinitrotoluene (2ADNT), 4-amino-2,6-dinitrotoluene (4ADNT), Gas chromatography mass spectrometry (GCMS)*

J Med Assoc Thai 2012; 95 (Suppl. 6): S154-S160
Full text. e-Journal: *http://jmat.mat.or.th*

2, 4, 6, -trinitrotoluene (TNT) is the most widely used military explosive because of its low melting point, its stability, low sensitivity to impact and friction and its relatively safe methods of manufacture[1]. TNT is the main chemical substance found on the ammunition production line. The toxicity of explosives has been widely known for many years. The effects of exposure to TNT have included upper respiratory tract irritation, aplastic anemia, toxic hepatitis, cataracts, hepatomegaly and liver cancer[2].

Detection of TNT in the blood or in urine is an indication of recent dermal, oral, or inhalation exposure. However, since TNT is rapidly metabolized, it may be difficult to determine trace amounts of the unchanged compound in blood or urine. The 4-amino-2, 6-dinitrotoluene (4ADNT) and 2-amino-2, 6-initrotoluene (2ADNT) are the main metabolites of TNT. They are eliminated in urine after conjugation into acid labile glucuronides[2]. In such cases, identification of major TNT metabolites such as 4ADNT and 2ADNT in the urine can be used as an indication of exposure. In one case of acute, primary dermal exposure to TNT, these two metabolites were present in the urine of exposed workers seventeen days after exposure[3]. This finding indicates that they can be used as indicators of not only recent, but also past, acute exposures to TNT. The objective of the present study is to determine the TNT, 2ADNT and 4ADNT levels in urine and the effect upon the health of workers.

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Material and Method

Chemicals and reagents

1,000 µg/ml TNT, 1,000 µg/ml 2ADNT and 1,000 µg/ml 4ADNT were purchased from Supelco, USA. Nitrobenzene was obtained from Carlo Erba, Italy.

Instrumentation

A gas chromatograph-mass spectrometer (Gas chromatograph, Hewlett Packard 5890 Series II) equipped with a mass selective detector (Hewlett

Packard 5972 Series) and integrator was used. The analytical condition was a DB-1701 capillary (30.00 m, 0.25 mm inner diameter). Helium was used as the carrier gas at a flow-rate of 1 ml/min. The GC condition for analysis of TNT, 2ADNT and 4ADNT was set as follows: injector temperature, 260°C; oven temperature, initial temperature of 80° C; then ramped at 30°C/min to 200°C and ramped at 5°C/min to 250°C and held for 8 min; ramped at 30°C/min to 270°C and maintained for 2 min; detector temperature, 280°C. The mass spectrometer used was a HP 5972 (Hewlett-Packard) at an ionization energy of 70 eV, set to selected ion monitoring mode, with a dwell time of 100 ms/ion and with the electron multiplier at 2,800 V.

Preparation of solutions

Concentrations of 1, 5, 10, 15 and 20 mg/L of working standard solution in urine (TNT, 2ADNT and 4ADNT) were prepared by pipetting 10, 50, 100, 150 and 200 µl stock 100 mg/L of mixed standard of each chemical and diluting to 10 ml with pooled normal urine. 100 µl nitrobenzene (Internal standard) was dissolved in ethyl acetate to create a final concentration of 30 mg/xL nitrobenzene.

Calibration curves

The mixed standard of TNT, 2ADNT and 4ADNT of 1, 5, 10, 15 and 20 mg/L of urine was added concentrated hydrochloric acid (0.1 ml) and nitrobenzene (0.1 ml, IS). Then the solutions were incubated in a thermo block at 100°C for 30 min. The solution was cooled and then neutralized by a sodium bicarbonate solution (0.15 g/sample). Following the neutralization, double extraction with ethyl acetate (2 x 6 ml), followed by vortex mixing (2 min) and subsequent centrifugation (3,500 rpm, 3 min) were carried out. The obtained supernatants were evaporated to dryness under a nitrogen flow. The residue was reconstituted with ethyl acetate (50 µl) and then placed into 0.5-ml auto-sampler vials. One microliter was injected onto the GC-MS. The calculated relative peak area ratio (the peak area of TNT and its metabolites/the peak area of nitrobenzene) was plotted against the concentrations of TNT and its metabolites of three replicate determinations.

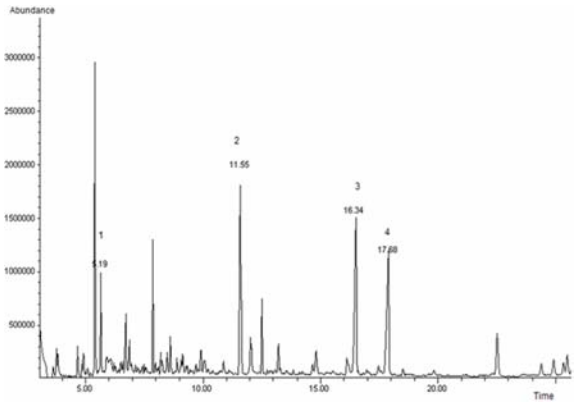

Fig. 1 Chromatogram presents peaks of nitrobenzene (1), TNT(2), 2ADNT(3) and 4ADNT(4) in pooled normal urine

Sample analysis

The analysis method was based on procedure published by Vorisek et al[4] and Bader et al[5] for analysis of TNT and TNT metabolites by GC-MS, with modification using nitrobenzene as an internal standard (IS). The calibration standards, quality control (QC), and blank and urine samples were analyzed at the same time. Concentrated hydrochloric acid (0.1 ml) and nitrobenzene (0.1 ml, IS) were added to the urine sample (2.5 ml) and the QC samples. Standard TNT, 2ADNT and 4ADNT in urine and the solutions were incubated in a thermoblock at 100°C for 30 min. The samples were cooled and then neutralized by a sodium bicarbonate solution (0.15 g/sample). Following the neutralization, the samples underwent double extraction into ethyl acetate (2 x 6 ml), then vortex mixing for two minutes, and then finally centrifugation at 3,500 rpm for three minutes. The obtained supernatants were evaporated to dryness under nitrogen, reconstituted in 50 µl of ethyl acetate and then placed in 0.5-ml auto-sampler vials. One microliter of each analytical sample was injected onto the GC-MS.

Detection limit

The concentrations of standard TNT, 2ADNT and 4ADNT solutions ranging from 0.2, 0.4, 0.6, 0.8 to 1.0 mg/L, respectively, were prepared in pooled normal urine. The solutions were analyzed in the same manner as the sample analysis. The detection limit was calculated following the method of the National Institute of Occupational Safety and Health (NIOSH)[6].

Accuracy and precision

The known concentrations of trinitrotoluene and its metabolites were prepared at concentrations of 2.5, 7.5 and 15 mg/L of urine. The solutions were analyzed in the same manner as sample analysis. The percent recovery and coefficient of variations of the three replicate determinations were calculated for

between-day assay.

Quality control

The pooled unexposed urines spiked at 2.5, 7.5 and 15 mg/L of TNT, 2ADNT and 4ADNT, then they were analyzed for three replications and for three days analysis. The mean and standard deviation of the three quality control samples were used for setting up the control chart to control the accuracy of the analysis.

Field application

The present study was a cross-sectional study in workers in the Artillery and Ammunition Plant in Lopburi. The present study was reviewed and approved by the Ethics Committee on Human Rights Related to Human Experimentation, Army Medical Department, Royal Thai Army No. SO19h/49.

The subjects attained for the present study were 80 workers divided into two groups according to an exposed group (n = 40) and a non-exposed group (n = 40). The exposed group consisted of workers who were exposed to TNT at the Filling TNT section in the Artillery and Ammunition Plant. The non-exposed group was comprised of workers who had no occupational exposure to TNT, such as administrative officers and personnel in other sections. The inclusion criteria of subjects consisted of adult males and females, aged 20-60 years old, from the TNT filling section. The exclusion criteria were those who had health records showing diseases or medical treatments for cardiovascular diseases, liver diseases, renal diseases, medical used and malignancy in liver or breast.

Sample collection and analysis

All subjects were interviewed using a questionnaire seeking general characteristics, work history, health status and personal protective equipment used. The urine samples of workers were collected post-shift in polyethylene bottles and kept at a temperature of 4°C during transportation. All samples were stored at -35°C in a refrigerator until analysis. Urine samples were analyzed following the sample analysis by GC-MS.

Statistical analysis

General characteristics of the present study subjects were displayed by percentage, median, mean and standard deviation. The relationship between urinary concentrations of TNT and its metabolites were illustrated using Spearman's rho correlation. The Chi-square test was used in examining the relationship between selected factors and TNT and its metabolites in urine samples from exposed workers.

Results

Biomarkers are useful in estimating the degree of exposure in cases where exposure is suspected or known.

Chromatograms of TNT, 2ADNT, 4ADNT

The pooled normal urine, with the addition of 5.0 mg/L of TNT, 2ADNT and 4ADNT, was analyzed and the chromatogram presented peaks of nitro-benzene, TNT, 2ADNT, 4ADNT at retention times of 5.19, 11.55, 16.34 and 17.68 min, r espectively (Fig. 1). Identification of trinitrotoluene, its metabolites and nitrobenzene (internal standard) was made from gas chromatography-mass spectrometry.

Standard curves of TNT, 2ADNT and 4ADNT in urine

The calibration curves for TNT, 2ADNT and 4ADNT were studied in the range of 1.0-20 mg/L of urine. Linear correlations were found between TNT, 2ADNT, 4ADNT and peak area ratios. The parameters of linear regression for TNT, 2ADNT, 4ADNT are given in Table 1.

Detection limit of the method

The detection limit of the method was studied in the range of 0.019-0.048 mg/L of urine. The detection limit of TNT, 2ADNT and 4ADNT were 0.048, 0.019 and 0.033 mg/L of urine.

Accuracy and precision

The pooled urine samples containing TNT, 2ADNT and 4ADNT at concentrations of 2.5, 7.5 and 15 mg/L of urine were used for determining accuracy

Table 1. Calibration parameters of TNT, 2ADNT and 4ADNT (relative peak area ratios vs. mg/L)

Compound	Slope	Intercept	Correlation coefficient (r)
TNT	0.0551	-0.0274	0.9981
2ADNT	0.0973	-0.0142	0.9996
4ADNT	0.1598	-0.0274	0.9989

and precision for a between-day assay. The recovery of analysis was in the range of 95.45 to 96.37% for TNT, 96.92 to 97.02% for 2ADNT and 97.87 to 98.02% for 4ADNT. The between-day assay coefficients of variation ranged from 0.1 to 0.95% for TNT, 0.06 to 0.41% for 2ADNT and 0.04 to 0.14% for 4ADNT.

Field application

General characteristics of workers

Exposed workers in the artillery and ammunition plant used TNT in the production process. 85% were male and 15% were female, with an average age of 44.98 (SD = 5.53) (Table 2). 75% of the non-exposed workers were male, while 25% were female with a combined average age of 50.30 (SD = 5.27), as shown in Table 2.

Health symptoms of workers

Subjects were interviewed about developing symptoms; the top five symptoms reported by workers exposed were nose irritation and throat irritation (70.0%), headaches and weakness (67.5%) and eye irritation (62.5%) as shown in Table 3. The top four symptoms reported by those not exposed were headaches (47.5%), weakness (30%), dizziness (27.5%) and an agitated mental state (15%). The symptoms reported by the non-exposed workers were much lower than those reported by the exposed workers.

Use of personal protective device

Workers of the present study used personal protective devices such as masks, cotton gloves, rubber gloves and chemical protective clothing. The results from the interview found that 100.0% of workers used personal protective devices except for one worker who did not use the chemical protective clothing.

Concentration of TNT and its metabolites in urine of workers

The results showed that TNT, 2ADNT and 4ADNT were not detected in urine of 3, 11 and 4 workers, respectively; whereas 37, 29 and 39 workers had median urinary TNT, 2ADNT and 4ADNT concentrations of 112.84 ranging from non-detectable (ND) to 1,833.81, 11.66 ranging from ND to 360.89 and 19.95 ranging from ND to 314.28 mg/L, respectively (Table 4). The TNT and its metabolites were not detected in all non-exposed urines.

Table 2. Characteristics of exposed and non-exposed workers

Variable	Number (%)	
	Exposed group	Non-exposed group
Sex		
Male	34 (85)	30 (75)
Female	6 (15)	10 (25)
Age(year)		
< 35	2 (5)	0
35-40	6 (15)	1 (2.5)
41-45	14 (35)	6 (15)
> 45	18 (45)	33 (82.5)
Status		
Single	3 (7.5)	6 (15)
Married	36 (90)	31 (77.5)
Divorce	1 (2.5)	3 (7.5)
Alcohol intake	24 (60)	16 (40.0)
Cigarette smoking	17 (42.5)	12 (30.0)
Type of job		
Melting or filling TNT	11 (27.5)	0
Others	29 (72.5)	40 (100)
Duration of exposure (month)		
≤ 180	8 (45)	40 (100)
> 180	22 (55)	0
Clean-up your body at end of shift	39 (97.5)	23 (57.5)

Table 3. Reported health symptoms of exposed and non-exposed groups

Symptom	Number (%)	
	Exposed Group	Non-exposed group
Headaches	27 (67.5)	19 (47.5)
Dizziness	21 (52.5)	11 (27.5)
Vomit/Nausea	9 (22.5)	2 (5)
Weakness	27 (67.5)	12 (30)
Loss of concentration	6 (15)	1 (2.5)
Ataxia	5 (12.5)	0 (0.0)
Loss of equilibrium	5 (12.5)	2 (5)
Cramp	14 (35)	1 (2.5)
Agitated mental	11 (27.5)	6 (15)
Chest tightness	12 (30)	0 (0.0)
Skin irritation/Itching	19 (47.5)	1 (2.5)
Rash	17 (42.5)	1 (2.5)
Dry skin and inflammation	8 (20)	0 (0.0)
Eye irritation	25 (62.5)	0 (0.0)
Nose irritation	28 (70)	0 (0.0)
Throat irritation	28 (70)	0 (0.0)

Table 4. Concentration of trinitrotoluene and its metabolites in urine of exposed group

	Urinary concentration (mg/L)			
	n	Median	Mean (SD)	Range
TNT				
Non-detectable	3		-	-
Detectable	37	112.84	237.07 (366.97)	ND-1,833.81
2ADNT				
Non-detectable	11		-	-
Detectable	29	11.66	42.93 (74.68)	ND-360.89
4ADNT				
Non-detectable	4		-	-
Detectable	36	19.95	53.68 (69.42)	ND-314.28

The relationship between selected factors and trinitrotoluene and its metabolites in the urine of workers

It was found that the type of job, duration of exposure, alcohol intake, tobacco used and use of personal protective devices did not have an association with urine TNT and its metabolites.

The relationship between trinitrotoluene and its metabolites in urine samples

Simple linear regression was used to test the relationship between TNT and its metabolites in urine. There were significant correlations between TNT and 2ADNT in urine ($r = 0.700$, p-value < 0.001), TNT and 4ADNT in urine ($r = 0.783$, p-value < 0.001). As a result, it was concluded that TNT had greater association with the presence of 4ADNT than 2ADNT did.

Discussion

The analysis method

The analysis method was modified from Vorisek et al[4] and Bader et al[5] for the analysis of TNT and its metabolites by adding nitrobenzene as an internal standard. The detection limit of method for the analysis of TNT, 2ADNT and 4ADNT were 0.048, 0.019 and 0.033 mg/L of urine, respectively. When compared to the detection limit of the method used in the former

Table 5. Correlation coefficients of TNT and its metabolites in urine of exposed group

Variable	Statistics	2ADNT	4ADNT
TNT	Correlation coefficient	0.700	0.783
	p-value	< 0.001	< 0.001

studies, Bader et al[5] could detect TNT, 2ADNT and 4ADNT at concentrations of 2, 25 and 1 µg/L of urine while Vorisek et al[4] could detect 4ADNT at a concentration of 0.05 mg/L of urine. The recovery of analysis of TNT, 2ADNT and 4ADNT at concentrations of 2.5, 7.5 and 15 mg/L urine, respectively, were greater than 95% with a coefficient of variation of less than 1%. The accuracy and precision of the method was satisfactory.

Field application

General characteristics of workers

The average ages of the exposed workers and non-exposed workers were 44.98 and 50.30 years old, respectively. The exposed workers smoked cigarettes and drank alcohol to a greater degree than the non-exposed subjects.

Health symptoms of workers

Most of the exposed group suffered from several symptoms, such as nose, throat and eye irritation, headache, asleep and skin irritation. The result of the present study coincided with former studies reporting inertia, nausea, insomnia, somnolence, dizziness, and headache were more prevalent with the exposed workers than in control workers[7]. Letzel et al stated that the exposed subjects reported bitter taste, drowsiness, headache, burning eyes, and discoloration of the skin or the hair more frequently than did the non-exposed subjects[8].

Concentration of TNT and its metabolites in the urine of workers

The average urinary TNT, 2ADNT and 4ADNT concentrations found in exposed workers at the artillery and ammunition plant ranged from ND-1,833.81, ND-360.89 and ND-314.28 mg/L, respectively. When the present study is compared with the former Bader et al study[5], they detected urinary TNT, 4ADNT and 2ADNT in six samples ranging from 4 to 43 µg/L, 143 to 16,832 µg/L and 24 to 5,787 µg/L, respectively. Letzel et al reported that workers regularly exposed to ammunition containing TNT and DNT had urinary TNT, 2ADNT and 4ADNT concentrations ranging from ND -5.0, ND-1,464, ND-6,693.0 µg/L, respectively[8]. The current study found much higher urinary TNT, 2ADNT and 4ADNT concentrations in exposed workers than the former studies had done. Most workers always bathed after work.

The relationship between selected factors and trinitrotoluene and its metabolites in urine of workers

The present study did not find a significant relationship between TNT, 2ADNT, 4ADNT concentrations and the type of job, duration of exposure, alcohol intake, smoking and personal protective devices used. This could be due to the fact that the workers worked only 4 to 5 hours a day. They usually worked for ten days and then went on hiatus for 10 to 20 days. Moreover, the production process did not continue throughout the year.

The relationship between trinitrotoluene and its metabolites in urine samples

A significant relationship was found between TNT and 2ADNT in urine ($r = 0.700$, p-value < 0.001), TNT and 4ADNT in urine ($r = 0.783$, p-value < 0.001).

Recommendation

The plant was designed in the European style, which did not have sufficient windows to vent heat and TNT vapors outside. The ventilation system is not suitable for Thailand, which is located in a tropical zone. The plant needs to increase ventilation with appropriate fans and water shields in order to reduce TNT vapors and the ambient temperature. The workers should have a physical check-up and environmental monitoring should be ongoing to monitor the potential health hazards from TNT. The urinary screening test for TNT exposure should be analyzed with a TNT lab stick.

Potential conflicts of interest

None.

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การรับสัมผัสไตรไนโตรโทลูอีน และผลกระทบต่อสุขภาพของคนงานในโรงงานผลิตปืนและกระสุน

พรพิมล กองทิพย์ สมรรถ ปรีกลาง วิทยา อยู่สุข สุทธินันท์ ฉันท์ธนกุล

วัตถุประสงค์*: เพื่อวิเคราะห์ปริมาณ 2, 4, 6 ไตรไนโตรโทลูอีน (TNT) 2-อะมิโน-4, 6-ไดไนโตรโทลูอีน (2ADNT) และ 4-อะมิโน-2, 6-ไดไนโตรโทลูอีน (4ADNT) ในปัสสาวะ และผลกระทบต่อสุขภาพของคนงานในโรงงานผลิตกระสุนปืน*

วัสดุและวิธีการ*: ตรวจวัดปริมาณ 2, 4, 6 ไตรไนโตรโทลูอีน (TNT) และเมตาโบไลท์ในตัวอย่างปัสสาวะ จากคนงานผลิตกระสุนปืน 40 คน และคนทำงานออฟฟิศ 40 คน ด้วยวิธีแกสโครมาโตกราฟฟี แมสสเปคโตรเมทรี สัมภาษณ์กลุ่มตัวอย่างด้วยแบบสอบถาม*

ผลการศึกษา*: ค่ามีเดียนของ TNT และเมตาโบไลท์ของ TNT ที่ตรวจวัดได้เป็น TNT 112.84 มีค่าอยู่ระหว่างค่าที่ตรวจวัดไม่ได้ ถึง 1,833.81 มิลลิกรัม/ลิตร 2ADNT 11.66 มีค่าอยู่ระหว่างค่าที่ตรวจวัดไม่ได้ถึง 360.89 มิลลิกรัม/ลิตร และ 4ADNT 19.95 มีค่าอยู่ระหว่าง ค่าที่ตรวจวัดไม่ได้ถึง 314.28 มิลลิกรัม/ลิตร ปริมาณของ 2ADNT มีความสัมพันธ์กับปริมาณ TNT อย่างมีนัยสำคัญทางสถิติ ($r = 0.700$; p-value < 0.001) และ 4ADNT มีความสัมพันธ์กับปริมาณ TNT อย่างมีนัยสำคัญทางสถิติ ($r = 0.783$; p-value < 0.001) คนงานรายงานอาการระคายเคืองตา จมูกและลำคอ อ่อนเพลีย ปวดศีรษะมากกว่ากลุ่มที่ไม่ได้สัมผัส*

สรุป*: ปริมาณ TNT มีความสัมพันธ์กับปริมาณ 4ADNT และ 2ADNT คนงานที่รับสัมผัสบ่นว่ามีอาการระคายเคืองตา จมูก และลำคอ*