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DETECTION OF TNT AND ITS METABOLITES IN BODY FLUIDS OF LABORATORY ANIMALS AND IN OCCUPATIONAL EXPOSED HUMANS

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ABSTRACT

Metabolites of 2,4,6-trinitrotoluene (TNT) were found in the urine of rats and in the blood of rabbits fed with TNT, in the urine of rats exposed to TNT by skin absorption, and in the urine of TNT munition workers. Urine and blood extracts were analyzed by liquid-chromatography/mass spectrometry (LC/MS). The metabolites found included untransformed TNT, 2-amino-4,6-dinitrotoluene (2-A), 4-amino-2,6-dinitrotoluene (4-A), 2,4-diamino-6-nitrotoluene (2 4-DA) and 2,6-diamino-4-nitrotoluene (2,6-DA).

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INTRODUCTION

The metabolism of explosives in the human body and the analysis of explosives and their metabolites in body fluids are of great importance in several applications:

1. In the biomedical field, munition workers are exposed to explosives in various ways, such as contact and inhalation of dust and vapor. Part of the inhalated explosive finds its way into the gastro-intestinal tract.[1]

   2,4,6-Trinitrotoluene (TNT) has been the most commonly used explosive since World War I. The toxic action of TNT in humans may lead to toxic hepatitis and aplastic anemia, and to less serious medical problems such as gastritis, dermatitis and anemia.[2,3] Periodical analysis of the body fluids of personnel working in explosives manufacturing plants could reveal traces of explosives and their metabolites.

2. In the environmental field, improper disposal of obsolete explosives may cause serious contamination problems.[4] Trace detection of explosives and their metabolites in blood and urine of animals and humans in the disposal area may reveal the extent of its contamination.

3. In the forensic field, during the bomb-making process traces of explosives may penetrate into the body through the pores of the hands or by vapor inhalation, and be detected as metabolites in the blood or urine of the suspect.

We have focused our interest on the metabolites of TNT, because it is one of the most widely used explosives. The purpose of this research was to detect and identify urinary metabolites of TNT in laboratory animals and in occupationally exposed humans, using liquid chromatography/mass spectrometry (LC/MS) as the analytical technique.

EXPERIMENTAL

1. INSTRUMENTATION

The LC/MS system consisted of an HPLC interfaced to a magnetic sector mass spectrometer.[5] Two HPLC systems and LC/MS interfaces were used:

a) An Eldex A-30-S Pump, a Rheodyne 7125 Sample Injector and a Waters 441 UV detector, operated at 214 nm. The column was a Brownlee reversed-phase RP-8 (C_8) column, 10 cm x 4.6 mm I.D. Mobile phases were acetonitrile:water at various concentrations at a flow rate of 1 ml/min. The LC/MS interface was a Hewlett-Packard variable split type Direct Liquid Introduction (DLI) probe.

b) A Gilson 302 Pump (with 5S Head), a Rheodyne 7520 Micro Sample Injector and a Waters 441 UV detector with Microbore Cell, operated at 214 nm. The column was a Brownlee reversed-phase RP-8 (C_8) micro-column, 10 cm x 2.1 mm I.D. Mobile phases were acetonitrile:water and methanol:acetonitrile:water at various concentrations at flow

rates of 120-130 µl/min. The LC/MS interface was a home-built Henion-type[6] Direct Liquid Introduction probe.

The difference between the two systems is that in the first one only 1-2% of the HPLC effluent enters into the mass spectrometer ion source, while in the second system the total effluent enters into the ion source.

2. ANIMAL EXPERIMENTS

a) Rats were fed with doses of 20 mg TNT dissolved in 1 ml corn oil, using a stomach tube. Each rat was then housed in a metabolic cage for urine collection. Urine extracts were analyzed by LC/MS.[7]

b) Rabbits were fed with doses of 100 mg TNT dissolved in 3.5 ml corn oil. Blood samples (3 ml) were taken from the rabbits' ears 1, 3, 6 and 12 h after feeding. Serum extracts were analyzed by LC/MS.[8]

c) Patches containing a mixture of 20 mg TNT with 5 drops of glycerol were applied on a surface of 4 cm^2 of the skin of rats for 2 hours. The patches were then removed and rats were housed in metabolic cages for urine collection. Urine extracts were analyzed by micro-LC/MS.

3. ANALYSIS OF URINE OF HUMANS

The extracts of seven urine samples from TNT workers were analyzed by micro-LC/MS.[9] No information was available regarding the exact closeness of these workers with TNT. Therefore TNT could have been absorbed by inhalation, digestion and skin absorption.

RESULTS

Tables 1-4 show the obtained results.

TABLE 1

Metabolites found in the urine of rats fed with TNT.[7]

Metabolite	Amount of metabolite found in urine [ng/ml urine]		
	After 12 h.	After 24 h.	After 48 h.
TNT	250 - 500	50 - 150	-
2-A	650 - 1200	250 - 750	100 - 350
4-A	550 - 1350	200 - 1000	50 - 500
2,4-DA	7 - 12	4 - 8	1 - 7

TABLE 2

Metabolites found in the blood of rabbits fed with TNT.[8]

Metabolite	Amount of metabolite found in blood [ng/ml serum]			
	After 1 h.	After 3 h.	After 6 h.	After 12 h.
TNT	66 - 293	102 - 306	13 - 82	4 - 15
2-A	19 - 161	41 - 132	9 - 47	2 - 7
4-A	13 - 96	18 - 78	6 - 21	1 - 8

TABLE 3

Metabolites found in the urine of rats in TNT skin absorption experiment

Metabolite	Amount of metabolite found in urine [ng/ml urine]	
	After 6 h.	After 12 h.
TNT	0 - 12	-
2-A	13 - 34	0 - 12
4-A	4 - 24	0 - 16

TABLE 4

Metabolites found in the urine of seven TNT workers

Metabolite	Amount of metabolite found in urine [ng/ml urine]
TNT	11 - 278
2-A	3 - 2720
4-A	23 - 9650
2,4-DA	24 - 300
2,6-DA	6 - 83

CONCLUSIONS

1. The results of laboratory animal experiments indicate that TNT absorbed by ingestion and skin penetration - as reflected in urine, and by ingestion - as reflected in blood - is transformed into metabolites formed by reduction processes.
2. Similar reduction metabolites are absorbed in the urine of TNT munition workers, where TNT could have been absorbed through inhalation, digestion and skin absorption.
3. We recommend the use of LC/MS for the early detection of TNT metabolites in urine as an analytical screening method

for the assessment of occupational absorption of TNT in munition workers.

4. More experimental work has to be done on skin absorption of TNT before coming to any operative conclusions in forensic applications.

5. The sensitivity of detection of metabolites of TNT could be improved by one to two orders of magnitude by upgrading the LC/MS system to thermospray - LC/MS.[10]

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