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Analysis of nitroaromatic compounds in urine by gas chromatography–mass spectrometry for the biological monitoring of explosives

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Abstract

Organic nitrocompounds are the most frequently used constituents of explosives and some of them have been evaluated to be highly toxic or even carcinogenic. Human contact with explosives may originate from a variety of sources, including occupational exposure during the production of ammunition as well as environmental exposure due to the contamination of soil and ground water reservoirs on former military production sites and training areas. This paper describes two gas chromatography–mass spectrometry–selected ion monitoring methods for the determination of twelve nitroaromatic compounds in urine (nitrobenzene, 1,2-dinitrobenzene, 1,3-dinitrobenzene, 1,3,5-trinitrobenzene, 2-nitrotoluene, 3-nitrotoluene, 4-nitrotoluene, 2,4-dinitrotoluene, 2,6-dinitrotoluene, 2,4,6-trinitrotoluene, 2-amino-4,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene). The analytes are detectable in the lowest μg/l range, with imprecisions of 3–22% within series and 5–29% between series, depending on the compound of interest. Both procedures are rapid and relatively easy to perform and, therefore, are advantageous for the screening of occupationally or environmentally exposed persons. We analysed urine samples obtained from nine workers from an ammunition dismantling workshop and from twelve control persons. 2,4,6-Trinitrotoluene was detected in six samples at concentrations between 4 and 43 μg/l. The main metabolites of 2,4,6-trinitrotoluene, 4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene, were found in a concentration range from 143 to 16 832 μg/l and from 24 to 5787 μg/l, respectively. Nonconjugated aminodinitrotoluenes were present as varying percentages of the total amount. 2,4-Dinitrotoluene and 2,6-dinitrotoluene were found in two samples (2–9 μg/l). Nitroaromatics were not detectable in urine specimens from control persons.

Keywords: Military waste; Biomonitoring; Nitroaromatics;

1. Introduction

With recent international agreements on arms limitation, the disposal of military waste as well as the restoration of the armaments industry and training grounds has become an occupational-medical and environmental problem [1–3]. During the last decade, the fate of military waste and possible health hazards arising from contaminated areas has come under constant and controversial discussion, especially in Germany [4–7].

In contrast, the continuous disposal of old and

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surplus military ammunition has so far been given less attention, as quantitatively it represented only a minor problem. With the reunification of Germany, however, 300 kilotons of ammunition from the former East Germany were taken over [8]. This problem evoked questions concerning exposure and health risk assessment for persons dismantling military ammunition or living in the neighbourhood of contaminated areas. Since 1991, excess ammunition has been decomposed in mechanical workshops throughout Germany and subsequently burned in waste incinerators. Ambient air measurements of nitroaromatics are inadequate for the estimation of health risks. Nitrotoluenes are readily absorbed through the skin, resulting in a higher uptake than would be expected from the inhalative route alone [9]. To assess the internal burden of nitroaromatics, determination of both the unchanged compounds and their metabolites has to be considered.

The most widely distributed and best-known nitroaromatic compound used in explosives is probably 2,4,6-trinitrotoluene (TNT), which, since the beginning of the century, has been used in military explosives. Trinitrotoluene is classified in Germany as a substance that is suspected of being potentially carcinogenic for humans. The major metabolic pathway of TNT as well as its ultimate toxic principle is the formation of aromatic amines by reduction of one or more nitro groups (Fig. 1). 4-Amino-2,6-dinitrotoluene (4-ADNT) and 2-amino-2,6-dinitrotoluene (2-ADNT) are the main metabolites of TNT and they are eliminated in urine after conjugation to acid-labile glucuronides [9].

TNT is regarded as a key compound and ADNTs have often been analyzed in biological material by GC–MS from people that had been exposed to it. In a study among workers of a TNT production plant, the ratio between 4-ADNT, 2-ADNT and their parent compound, TNT, was found to be approximately 100:22:0.7 [10]. This result is consistent with other studies on the same subject [11–13], indicating that ADNTs are both diagnostically sensitive and specific biomonitoring parameters for TNT exposure. The adequacy of unchanged nitroaromatics for biological monitoring, however, has been rated differently.

To address the question of appropriate biomonitoring parameters for explosives and to evaluate the validity of metabolically unchanged nitroaromatics for this purpose, we established two specific, sensitive and reliable methods for the determination of (1) metabolically unchanged nitroaromatics and non-conjugated (free) aminonitrotoluenes in urine and (2) the total amount of the TNT metabolites, 4-ADNT and 2-ADNT. In a pilot study on occupational exposure to explosives during the dismantling of ammunition, both methods were applied to the analysis of urine samples obtained from exposed workers and from controls.

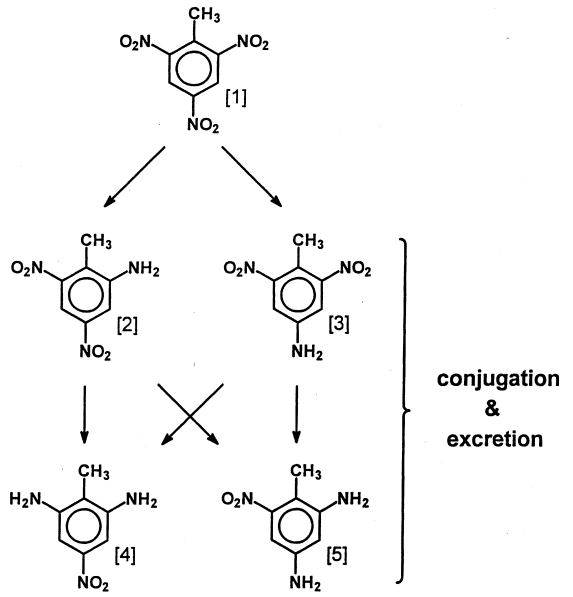

Fig. 1. Basic metabolism of trinitrotoluene (TNT) [(1) trinitrotoluene, (2) 2-amino-4,6-dinitrotoluene, (3) 4-amino-2,6-dinitrotoluene, (4) 2,6-diamino-4-nitrotoluene and (5) 2,4-diamino-6-nitrotoluene).

2. Materials and methods

2.1. Chemicals

A mixture of nitroaromatics in methanol (nitrobenzene, 1,2-dinitrobenzene, 1,3-dinitrobenzene, 1,3,5-trinitrobenzene, 2-nitrotoluene, 3-nitrotoluene, 4-nitrotoluene, 2,4-dinitrotoluene, 2,6-dinitrotoluene, 2,4,6-trinitrotoluene, 2-amino-4-nitrotoluene, 2-amino-6-nitrotoluene, 2-amino-4,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene, nitroguanidine, octogen, hexogen, tetryl, picric acid, nitropenta (concentration, 10 mg/l) was from Ehrenstorfer (Augsburg, Germany), $^{13}C_6$-Nitrobenzene (99%) was from Cam-

bridge Isotope Laboratories (Andover, MA, USA), 4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene (both of 99% purity) were from Promochem (Wesel, Germany). 4-Amino-3,5-dinitrotoluene (99%), and 2-(*N*-morpholino)ethanesulfonic acid (MES), pentafluoropropionic anhydride (PFPA) and pyridine (all of the highest analytical grade) were from Sigma-Aldrich (Deisenhofen, Germany). Acetic acid (conc.), ethanol, diethyl ether, hydrochloric acid (25%), isooctane, methanol, *ortho*-phosphoric acid, potassium phosphate trihydrate, sodium chloride, sodium hydroxide, sodium sulfate, sulfuric acid (conc.) and toluene were all of the highest available analytical grade and were from Merck (Darmstadt, Germany).

To make 10 *M* NaOH, 400 g of sodium hydroxide were dissolved in 1000 ml of bidest. water. To make 0.25 *M* MES, 53.6 g of 2-(*N*-morpholino)ethanesulfonic acid were dissolved in 1000 ml of bidest. water. The pH was adjusted to six by the addition of 10 ml of NaOH. A 0.01-*M* phosphate buffer was made by dissolving 5.4 g of potassium phosphate trihydrate in 2000 ml of bidest. water. The pH was adjusted to eight by the addition of 750 μl of *ortho*-phosphoric acid.

2.2. Standards

2.2.1. Method A

This method was used for the determination of nitroaromatics and free aminodinitrotoluenes. A 99.5-ml volume of pooled urine from persons without known exposure to nitroaromatics and other explosive constituents was spiked with 500 μl of the nitroaromatics mix to yield a stock solution with a concentration of 50 μg/l of each compound. Calibration standards were prepared in a range between 1 and 50 μg/l by dilution with pooled urine. For evaluation of the analytical reliability criteria, 99.9 ml of pooled urine were spiked with 100 μl of the nitroaromatics mix (concentration, 10 μg/l) and this was divided into 5 ml aliquots. All standards were kept frozen.

2.2.2. Method B

This method was used for the determination of total aminodinitrotoluenes). A 25-mg amount of 4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene was dissolved in 25 ml of ethanol (concentration, 1000 mg/l). The solution was diluted either tenfold with bidest. water to give a concentration of 100 mg/l or 200-fold to give a concentration of 5 mg/l. Calibration standards were prepared from these stocks and from pooled urine from controls (range, 5–20 000 μg/l). Quality control material was made from spiked pooled urine samples (concentration, 15 μg/l). The standards and the control material were stored at −27°C.

2.3. Internal standards

Method A: A 100-μl volume of $^{13}C_6$-nitrobenzene (δ=1.20 g/ml) was dissolved in 19.9 ml of methanol (concentration, 6 g/l) and further diluted in two steps (1:200 and 1:20) to a final concentration of 1.5 mg/l in methanol. The solution was stored at 4°C.

Method B: A 25-mg amount of 4-amino-3,5-dinitrotoluene was dissolved in 50 ml of ethanol (concentration, 500 mg/l). A 1-ml volume of the stock solution was diluted by a factor of 100 with bidest. water to a final concentration of 5 mg/l. The solution was stored at 4°C.

2.4. Study subjects and sample collection

Urine samples (50–100 ml) were supplied by nine workers (seven female, two male) from an ammunition dismantling workshop in Saxonia, Germany, who were involved in the disposal of various types of artillery shells, aircraft bombs and hand grenades. The urine was collected postshift in polyethylene bottles and kept at a temperature of 4°C during transport. Additionally, urine was collected from twelve people of the laboratory staff who did not have known exposure to explosives or nitroaromatics. All samples were stored frozen at −27°C.

2.5. Analysis of nitroaromatics and free monoaminonitrotoluenes (method A)

After equilibration to room temperature and shaking of the samples, 5 ml of urine were transferred into a 10 ml screw-capped glass vial and spiked with 100 μl of internal standard A. A 1500-mg amount of sodium chloride, 500 μl of hydrochloric acid and 1 ml of isooctane were added to each vial and the samples were extracted for 1 min by vigorous

shaking on a laboratory mixer. The vials were centrifuged for 5 min at 3000 *g*. An 800-μl volume of each organic layer was transferred into a 1.5-ml glass vial and concentrated to a final volume of 100 μl under a gentle stream of nitrogen. A 1-μl volume of each sample was analyzed by GC–MS (Fig. 2). The standards and control material were treated likewise.

2.6. Analysis of total monoaminodinitrotoluenes (method B)

A 5-ml volume of each urine sample was spiked with 100 μl of internal standard B and 1 ml of sulfuric acid. Hydrolysis of acid-labile conjugates was achieved by heating the mixture for 1 h at 80°C. Following equilibration to room temperature, 50 μl of acetic acid were added and the samples were cooled in an ice bath before 3.7 ml of 10 *M* NaOH were added (3×1 ml and 1×400 μl) followed by 8 ml of MES buffer and another 300 μl of 10 *M* NaOH. The pH of the hydrolyzed urines should be higher than 5.5 but below 7.0. After the addition of 5 g of sodium chloride, the urine samples were extracted three times with 5 ml of diethyl ether. The organic layers were combined with 2 ml of isooctane in a 20-ml screw-capped glass vial, concentrated to a volume of 2 ml under a gentle stream of nitrogen and finally dried for 30 min by the addition of 1000 mg of sodium sulfate. The samples were then transferred to a 20-ml screw-capped glass vial, while the sodium sulfate was washed twice with 2 ml of isooctane. The layers were combined before 50 μl of pyridine (dried over sodium sulfate) and 200 μl of pentafluoropropionic anhydride were added. Derivatization was achieved by tempering the vials for 1 h at 65°C and for 16 h at 37°C in a water bath. To remove excess pentafluoropropionic acid, the samples were cooled to room temperature and extracted once with 10 ml of phosphate buffer for 10 min on a laboratory mixer. The layers were then separated by centrifugation at 1000 *g* for 5 min. The organic solvent was transferred into a 5-ml glass vial containing 200 μl of toluene, dried with 500 mg of sodium sulfate and concentrated to a final volume of 200 μl under a gentle stream of nitrogen. A 1-μl volume of each sample was then analyzed by GC–MS. The same procedure was applied to the calibration standards and the control material.

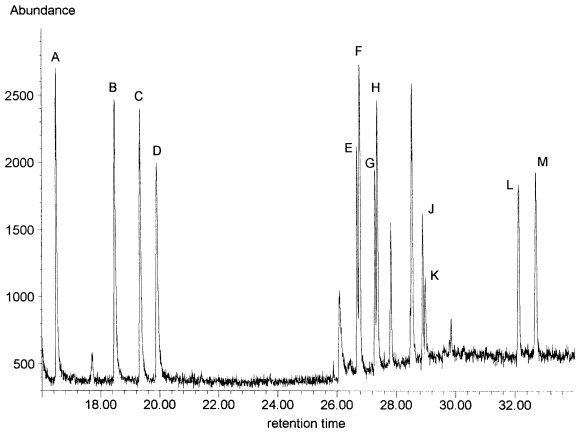

Fig. 2. GC-MS chromatogram (scan 50-250 *m/z*, method A) of a nitroaromatics standard (concentration, 1 mg/l) [(A) nitrobenzene, (B) 2-nitrotoluene, (C) 3-nitrotoluene, (D) 4-nitrotoluene, (E) 1,3-dinitrobenzene, (F) 2,6-dinitrotoluene, (G) 1,2-dinitrobenzene, (H) 2,4-dinitrotoluene, (J) 2,4,6-trinitrotoluene, (K) 1,3,5-trinitrobenzene, (L) 2-amino-4,6-dinitrotoluene and (M) 4-amino-2,6-dinitrotoluene).

2.7. GC–MS conditions

The GC–MS system used consisted of a gas chromatograph HP 5890 series II plus (Hewlett-Packard, USA), a DB-17 fused silica capillary column (60 m×0.25 mm, 0.3 μm film; J&W Scientific, USA) and a liquid sample injector HP 7673 (Hewlett-Packard). A sample volume of 1 μl was used in the splitless mode. Helium 5.0 was used as the carrier gas at a flow-rate of 1 ml/min (constant), an injector temperature of 260°C and a transfer line temperature of 280°C. The oven program for method A was as follows: Initial column temperature, 95°C, increasing at 3°C/min to 200°C, isothermal for 10 min, increasing at 25°C/min to 275°C and isothermal for 40 min. The oven program for method B was as follows: Initial column temperature, 60°C, increasing by 5°C/min to 180°C, increasing by 30°C/min to 270°C and isothermal for 18 min. The mass spectrometer used was a HP 5972 (Hewlett-Packard) at an ionization energy of 70 eV, in selected ion monitoring mode, with a dwell time of 100 ms/ion and with the electron multiplier at 2800 V. The analytes were identified by their retention times and two characteristic mass fragments (Tables 1 and 2).

Table 1
Retention times (t_R), molecular masses (M_r) and selected ions for the GC–MS analysis of nitroaromatics and free aminodinitrotoluenes in urine (method A)

Compound	t_R (min)	M_r (g/mol)	SIM ions (m/e)	
ISTD	16.5	129	129	83
NB	16.5	123	123	77
2-NT	18.5	137	120	137
3-NT	19.3	137	137	120
4-NT	19.9	137	137	120
1,3-DNB	26.7	168	168	92
2,6-DNT	26.8	182	165	89
1,2-DNB	27.3	168	168	92
2,4-DNT	27.4	182	165	89
TNT	28.9	227	210	89
TNB	29.0	213	213	120
2-ADNT	32.1	197	197	180
4-ADNT	32.7	197	197	180

ISTD, internal standard.

3. Results and discussion

The methods we describe in this paper for the analysis of explosives and their metabolites in urine were based on procedures published by the Deutsche Forschungsgemeinschaft [14,15] for the determination of nitroaromatics in plasma and of aromatic amines in body fluids by GC–ECD. To investigate exposure to explosive constituents, we adapted the original methods to the urine matrix, to extend them to the compounds of interest, and to evaluate the analytical reliability criteria of the modified methods. We chose urine instead of plasma because the nitroaromatics and their metabolites are eliminated renally [11,16–19] and because the method of sampling is easier and preferable for the study subjects.

Table 2
Retention times (t_R), molecular masses (M_r) and selected ions for the GC–MS analysis of PFPA derivatives of total aminodinitrotoluenes in urine (method B)

Compound	t_R (min)	M_r (g/mol)	SIM ions (m/e)	
ISTD	42.5	343	297	251
2-ADNT	43.1	343	326	206
4-ADNT	44.6	343	326	224

ISTD, internal standard.

Additionally, we had to improve the detection limits, as other studies [11,20,21] indicated that only small percentages of nitroaromatics are excreted in a metabolically unchanged form. For the biomonitoring of low-dose and environmental exposure, we had to expect the lowest concentrations of analytes.

In the case of method A, we modified the original procedure by starting with more biological material (5 ml of urine instead of 1 ml of plasma) and by concentrating the extract. The sensitivity of a mass spectrometric detector for nitroaromatics is comparable to that of ECD, but it is more specific and the changes we introduced to the sample preparation therefore improved the detection limits considerably (e.g. from 15 to 0.5 μg/l for nitrotoluene, from 10 to 0.5 μg/l for nitrobenzene and from 2 to 0.5 μg/l for dinitrotoluenes). Additionally, we found that saturation of the urine with sodium chloride and acidification increased the recovery of analytes with more than one nitro group.

Table 3 summarizes the analytical reliability criteria for the analysis of nitroaromatics in urine by GC–MS. The compounds can be classified into three subgroups with different detection limits and reliability: The first group contains nitrobenzene, the isomeric nitrotoluenes and dinitrotoluenes and has a detection limit of 0.5 μg/l for each compound, within-series imprecisions of below 10% and between-series imprecisions of below 15%. All of the analytes had an overall recovery rate of about 70–80%. The second group, with a recovery of 50–60%, includes the dinitrobenzenes and 2,4,6-trinitrotoluene, with detection limits of 1 and 2 μg/l, respectively, and imprecisions of about 10% within series and below 15% between series. The worst detection limits and reliability were observed for 1,3,5-trinitrobenzene (detection limit, 10 μg/l; imprecision, 17% within series and 24% between series) and the free aminodinitrotoluenes (detection limit, 25 μg/l; imprecision within series, 22% (2-ADNT) and 12% (4-ADNT); imprecision between series, 29% (2-ADNT) and 25% (4-ADNT). The calibration curves were linear at least up to 1000 μg/l for each analyte.

These results clearly demonstrate that the analytical reliability of the method is closely related to the hydrophilic properties of the molecule of interest, e.g. its tendency to form hydrogen bonds and, thus, the number of nitro groups. As expected, the analysis

Table 3
Analytical reliability criteria

Compound	Detection limit (μg/l)	Imprecision within series (%)	Imprecision between day (%)	Recovery (%)
NB	0.5	3	5	78
2-NT	0.5	5	5	73
3-NT	0.5	9	11	75
4-NT	0.5	8	10	75
2,6-DNT	0.5	6	8	71
2,4-DNT	0.5	9	13	74
1,3-DNB	1	11	13	61
1,2-DNB	1	8	10	55
TNT	2	13	14	58
TNB	10	17	24	44
2-ADNT	25	22	29	nd
4-ADNT	25	12	25	nd
2-ADNT[a]	4	13	8	nd
4-ADNT[a]	1	8	17	nd

[a] According to method B.
nd, not determined.
Detection limits are defined as the threefold signal-to-noise ratio.
Imprecision within series was calculated from $n=9$ repeated sample preparations on one day (concentration, 10 μg/l).
Imprecision between days was calculated from single sample preparations on six different days (concentration, 10 μg/l).
Recovery was calculated from a comparison of a standard (10 μg/l) worked-up in triplicate and a corresponding methanol dilution of the nitroaromatics standard.

of nitroaromatics with two or more nitro groups suffers from incomplete and varying extraction rates from the urine matrix with the nonpolar solvent isooctane. Although this aspect might be optimized by using a different extraction solvent, isooctane leaves the bulk of polar compounds in the urine matrix, ensuring a minimum amount of interference and analytical background. In conclusion, the reliability of the method is adequate and justifies its use for the analysis of nitroaromatics in the lower μg/l range. Sample preparation is relatively easy, inexpensive and rapid. Except for the GC–MS system, no sophisticated material is needed and large sample numbers can be treated in a single series, rendering the method particularly suitable for routine monitoring or screening.

Some important explosive constituents, however, could not be analyzed with the above described method, especially those of the nitramine type, such as hexogen or octogen. This is probably due to losses of analytes during the extraction or to thermal decomposition in the injector system of the gas chromatograph [22,23]. An on-column injector or a cooled inlet system should be capable of solving the latter problem.

Analysis of the total amount of the TNT metabolites 4-ADNT and 2-ADNT using method B is based on the release of acid from conjugates and a subsequent derivatization step. Although ADNTs are often not derivatized prior to analysis [9,10], interfering compounds are more effectively separated during the sample work-up and better detection limits are achieved this way. Additionally, aminodinitrotoluenes are susceptible to oxidation and a derivatization step stabilizes the analytes. Some aspects of the original procedure had to be carefully optimized in order to obtain sufficient detection limits and analytical reliability criteria. The first is adjustment of the pH of the urine after the hydrolysis of conjugates. While simple aromatic amines are usually extracted into an organic solvent from alkalized aqueous samples, we found that aminodinitrotoluenes require a pH value of between five and seven in order to get reliable extraction yields. We introduced a MES buffer system to maintain this range after the addition of a sodium hydroxide

solution. Another important step involved the handling of the isooctane extracts. First of all, the organic layers have to be carefully dried with sodium sulfate before the derivatization of the analytes with pentafluoropropionic anhydride (PFPA) is commenced. PFPA is very susceptible to hydrolysis and traces of water will immediately decrease the derivatization yield. Furthermore, the sodium sulfate has to be intensively washed and extracted with isooctane, to remove adsorbed analytes. A third important aspect of the procedure involves the extraction of excess anhydride and acid from the isooctane after the derivatization step.

The pentafluoropropionic acid derivatives of aminodinitrotoluenes are relatively polar and can be easily extracted into aqueous media. Therefore, every washing step leads to a significant loss of analytes. As a consequence, a single extraction step with a larger volume of buffer was used instead of a repeated procedure with smaller volumes.

The detection limits for this method reach the low μg/l range (Table 3). The calibration curves for both analytes are linear up to 20 mg/l. The imprecision within series is about 13% for 2-ADNT and 8% for 4-ADNT, while the imprecision between series varies between 8% (2-ADNT) and 17% (4-ADNT). These results are satisfactory with respect to the relatively arduous sample treatment and the susceptibility of aromatic amines to oxidation, promoted e.g. by the alkaline environment during some of the work-up steps.

In a pilot study, we applied the above described methods to the analysis of urine samples obtained from twelve controls and from nine workers of an ammunition delaboration workshop (Fig. 3). The controls revealed no physiological background of any nitroaromatic compound, but we found 2,4,6-trinitrotoluene in six of nine urine samples of the TNT-exposed workers, at concentrations ranging between 4 and 43 μg/l (mean, 16 μg/l; standard deviation, 16 μg/l; Table 4). 2,4-Dinitrotoluene and 2,6-dinitrotoluene were detected in two of the TNT-containing samples at concentrations of 2 and 4 μg/l (2,4-DNT) and 4 and 9 μg/l (2,6-DNT).

These results point to exposure to different types of explosives with varying contents of nitroaromatics. TNT is the main constituent of most military explosives and, therefore, it is apparent in most urine samples, while DNTs are used only to a minor extent, if at all, and more often occur only as contaminants of TNT [24]. No other nitroaromatics were found in the urine samples from the exposed workers. These compounds were not expected in the samples anyway as they are rarely used in explosives and are assumed to be rapidly metabolized upon uptake [18].

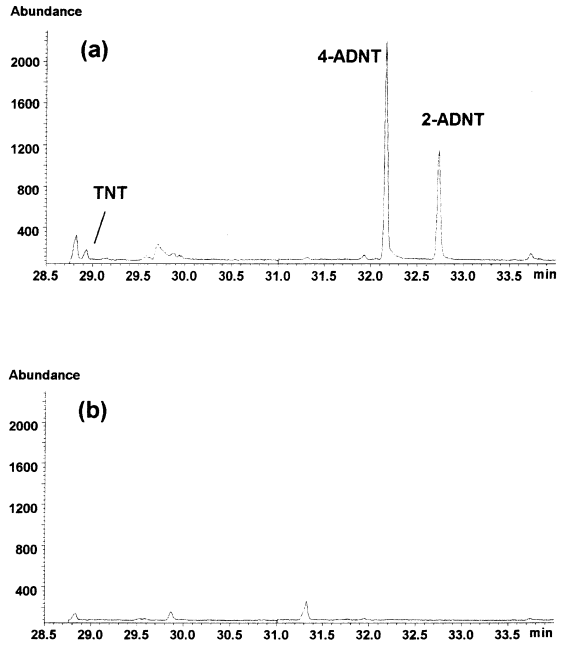

Fig. 3. Analysis of nitroaromatics and free aminodinitrotoluenes according to method A. GC–MS–SIM chromatograms of (a) an exposed worker (TNT, 8 μg/l; 4-ADNT, 3455 μg/l; 2-ADNT, 3429 μg/l) and (b) a control person.

Aminodinitrotoluenes were not detected in the control urines but were detected in those from the workers (Fig. 4, Table 4). The results for total 4-ADNT ranged from 143 to 16 832 μg/l, with a mean value of 7916 μg/l (standard deviation, 6513 μg/l), while 2-ADNT was detected at concentrations between 24 and 5787 μg/l (mean, 2157 μg/l; standard deviation, 2248 μg/l). A statistically significant correlation ($P < 0.05$) was observed between the two isomers (Fig. 5), which had to be expected as TNT is their common precursor. Free ADNTs were found in seven of the nine samples and correlated with the corresponding total amount of ADNTs in the case of 2-ADNT ($y = 0.48x - 110$,

Table 4
Biomonitoring results from the pilot study

Subject	2,4-DNT	2,6-DNT	TNT	2-ADNT (free)	2-ADNT (total)	4-ADNT (free)	4-ADNT (total)
1	–	–	43	2298	4939	2351	16488
2	–	–	–	18	71	92	1141
3	–	–	–	–	24	–	337
4	–	–	28	389	1397	552	7790
5	4	9	8	3439	5787	3455	11943
6	–	–	5	335	1724	706	6528
7	2	4	7	1208	1196	3253	10039
8	–	–	–	–	24	–	143
9	–	–	4	878	4252	1510	16832

All concentrations are given in μg/l.

$r=0.856$, $P<0.05$) and in the case of 4-ADNT ($y=0.14x+270$, $r=0.590$, $P=0.16$).

The relationship between the average values of 4-ADNT, 2-ADNT and TNT was 100:27:0.1. Considering the small number of subjects in our study, this ratio is in good agreement with the results obtained in the study by Ahlborg et al. [10] (100:22:0.7, $n=41$). These authors found average concentrations of 68 μg/l for 4-ADNT (maximum value, 573 μg/l), 15 μg/l for 2-ADNT (maximum value, 171 μg/l) and 0.5 μg/l for TNT (maximum value, 7 μg/l). A similar relationship between 4-ADNT and 2-ADNT (100:31) has previously been reported in blood samples from 93 people who were exposed to TNT [13].

The ambient air concentration of TNT in the study by Ahlborg et al. [10] was 0.1–0.5 mg/m^3. No air

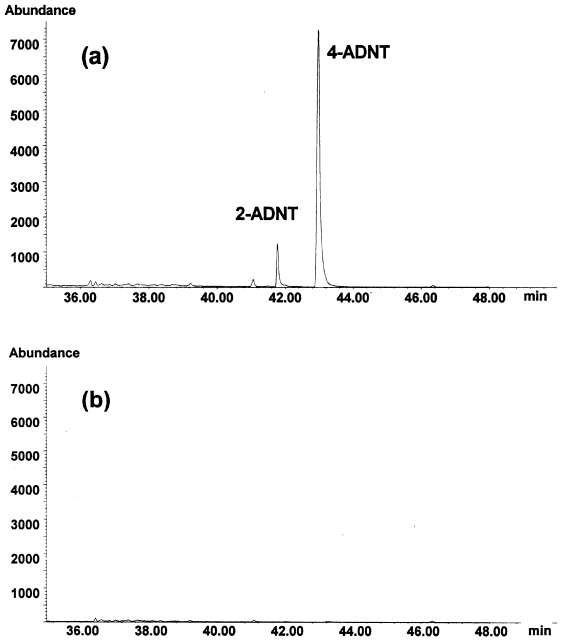

Fig. 4. Analysis of total aminodinitrotoluenes according to method B. GC–MS–SIM chromatograms of (a) an exposed worker (4-ADNT, 406 μg/l; 2-ADNT, 181 μg/l) and (b) a control person.

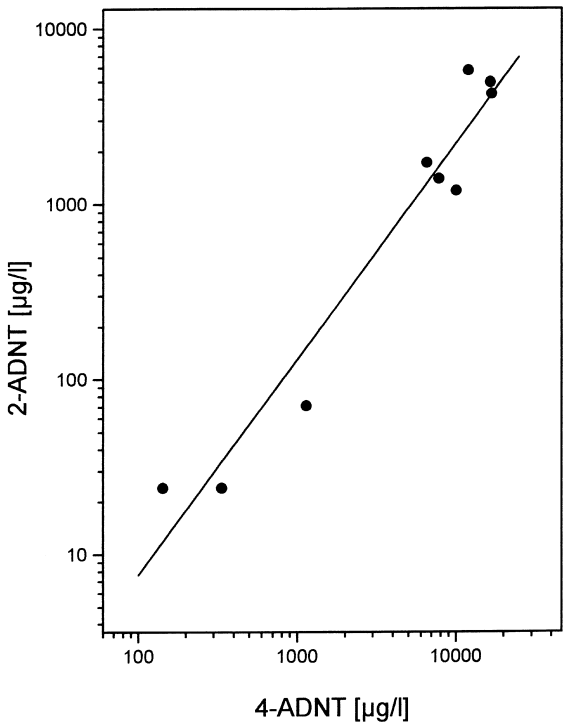

Fig. 5. Correlation between the TNT metabolites 4-ADNT and 2-ADNT in urine samples from exposed workers (linear regression: $y=0.3x-238$, $r=0.976$, $P<0.05$, $n=9$).

measurements were performed in the pilot study we describe, but previous investigations during technical surveillance in the plant revealed a range of 0.01–1 mg/m^3 for TNT in air. Although the exposure situation is comparable between the Ahlborg study and ours, we found about 100-fold elevated concentrations of ADNTs and fifteenfold elevated TNT concentrations in the urine samples. This result may be a consequence of enhanced dermal uptake by the people in our study as the dismantling of ammunition was dominated by abrasive and dust-developing worksteps.

Due to the relatively small number of people in our pilot study, the results have to be confirmed by carrying out a study on a larger scale. They emphasize, however, three aspects for the future biomonitoring of explosives. First of all, the analytical methods we established for the analysis of nitroaromatics and their monoamino metabolites are suitable for the routine monitoring of explosive-specific compounds. Sufficient sensitivity, specificity and analytical reliability was achieved for most compounds. Secondly, the aminodinitrotoluenes seem to be the most appropriate parameters for biomonitoring purposes. Unlike TNT itself, the metabolites were detected in every urine sample from people who were exposed to explosives. The lowest value for 4-amino-2,6-dinitrotoluene (143 μg/l) was more than 100 times above the detection limit of about 1 μg/l for this compound, pointing out the high diagnostic specifity of 4-ADNT. The last aspect to mention is the analysis of dinitrotoluenes. Unmetabolized DNTs were detected in only two samples. As a consequence, this parameter seems to be of limited significance for the biomonitoring of DNT exposure. The monitoring of DNT metabolites, e.g. dinitrobenzoic acids, as suggested by other authors [20], will be considered in future studies.

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