ORIGINAL ARTICLE

Exposure to nitroaromatic explosives and health effects during disposal of military waste

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Occup Environ Med 2003;**60**:483–488

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Accepted 11 October 2002

Aims: To investigate the exposure to dinitrotoluene (DNT) and trinitrotoluene (TNT) and the resulting effects in workers which occur during the disposal of military waste.
Methods: Eighty two employees from a mechanical plant in Germany were studied, of whom 51 were regularly exposed to ammunition containing TNT and DNT, 19 occasionally, and 12 not at all.
Results: Air analyses yielded maximum concentrations of 20 µg/m^3 for 2,4-DNT and 3250 µg/m^3 for 2,4,6-TNT, respectively. The maximum concentrations in the urine of workers regularly exposed amounted to 5.0 µg/l of 2,4,6-TNT, 1464.0 µg/l of 2-amino-4,6-dinitrotoluene, 6693.0 of µg/l 4-amino-2,6-dinitrotoluene, 2.1 µg/l of 2,4-DNT, 95.0 µg/l of 2,4-dinitrobenzoic acid, and 3.6 µg/l of 2,6-DNT. There was a highly significant linear correlation between the urinary concentrations of the two main metabolites of TNT, 2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene. In 63 persons TNT or DNT or metabolite concentrations above the analytical detection limit were found in urine. These persons reported more frequently symptoms like bitter taste, burning eyes, and discoloration of the skin and hair than persons (n = 19) without detectable TNT and/or DNT exposure.
Conclusion: During the disposal of military waste containing relevant TNT and DNT, exposure can occur of occupational-medical relevance. Biological monitoring is suitable for the early detection of possible adverse effects at workplaces exposed to TNT. Protective measures should be improved, together with adequate occupational-medical surveillance of persons exposed to nitroaromatic explosives. Further studies are necessary to exclude possible long term effects.

The disposal of military waste has recently become an important problem from an occupational-medical and environmental-medical point of view. On the territory of the former German Democratic Republic alone, approximately 300 000 tons of ammunition from stores had to be destroyed or dismantled after the reunification of Germany.[1] In addition, as a result of disarmament agreements and aging processes there is always ammunition in need of adequate disposal. Important components of military ammunition, particularly in earlier years, were the nitroaromatic dinitrotoluenes and trinitrotoluenes.

Technical grade dinitrotoluene (DNT) is mainly a mixture of 2,4-DNT (approx. 71–77%) and 2,6-DNT (approx. 18–20%). DNTs are well absorbed via the skin, the respiratory tract, and the gastrointestinal tract. In the urine of occupationally exposed workers 2,4-dinitrobenzoic acid (2,4-DNBA) was identified as the main metabolite of both pure 2,4-DNT and of technical grade DNT.[2] Additionally, methaemoglobin (MetHb) can be formed in the blood in a dose dependent manner after short term exposure. Furthermore, non-specific general symptoms such as headache, irritation of the mucous membranes, nausea, and vomiting are observed.[3 4] Exposure to dinitrotoluenes may lead to discoloration of the skin and hair. DNTs have been found to be carcinogenic in animal experiments. Stayner and colleagues[5] observed an increased risk of hepatobiliary carcinomas in workers exposed to DNTs in the production of ammunition. These results could not, however, be confirmed in any other study until now. For reasons of disease prevention DNTs and their isomeric mixtures are classified at present in category 2* by the "Deutsche Forschungsgemeinschaft" (DFG). In addition, the increased occurrence of cardiovascular changes after long term exposure to DNTs is under discussion.[6 7] At present there is no threshold limit value for DNT mixtures in Germany. For 2,6-dinitrotoluene a technical exposure limit (TRK value) was set to 0.05 mg/m^3.[8]

Technical grade trinitrotoluene (TNT) is a mixture of isomers. The main component with a 95% share is 2,4,6-TNT. Trinitrotoluene is well absorbed by the body via the skin, respiratory tract, and the gastrointestinal tract. It is metabolised, for example by nitroreductases of the liver, to form the two main metabolites, 2-amino-4,6-dinitrotoluene (2-ADNT) and 4-amino-2,6-dinitrotoluene (4-ADNT). The absorbed TNT and its metabolites are mainly excreted with the urine. After occupational exposure to TNT, dose dependent irritation of the skin and mucous membranes, liver function disturbances, disturbances of the erythrocytes up to aplastic anaemia, and discoloration of the skin and hair have been observed.[3 9] There are reports of haemolytic anaemia in persons exposed to TNT with a glucose-6-phosphate-dehydrogenase deficiency.[10 11] In addition, in single studies an increased incidence of cataracts of the eyes,[12] and changes in the male spermatozoa[13] were observed after exposure to TNT. Ahlborg and colleagues[14] described increased mutagenic effects caused by TNT. It has not been conclusively decided whether TNT can cause cancer. For reasons of disease prevention 2,4,6-TNT and its isomers in

Abbreviations: 2-ADNT, 2-amino-4,6,-dinitrotoluene; 4-ADNT, 4-amino-2,6,-dinitrotoluene; 2,4-DNBA, 2,4-dinitrobenzoic acid; DNT, dinitrotoluene; TNT, trinitrotoluene

*Substances that are considered to be carcinogenic for man because sufficient data from long term animal studies or limited evidence from animal studies substantiated by evidence from epidemiological studies indicate that they can make a significant contribution to cancer risk.

Table 1 Study group

	Urinary TNT, DNT, and metabolite concentration	
	<DL	>DL
Number	n=19	n=63
Sex		
Female	n=4	n=14
Male	n=15	n=49
Age (years)		
Median	46	45
Range	32–60	26–57
Duration of exposure (months)		
Median	51	59
Range	0–59	0–59
Anamnestic data about exposure		
None	n=7	n=5
Occasional	n=5	n=11
Regular	n=4	n=47
Smoking habits		
Never smoked	n=7	n=29
Ex-smoker	n=5	n=10
Smoker	n=7	n=24
Current alcohol consumption (g/day)		
Median	20	20
Range	0–55	0–140

DL, analytical detection limit.

technical grade mixtures are therefore classified in Germany in category 3b.** The maximum concentration at the workplace (MAK value) for TNT at present in Germany is 0.1 mg/m^3.[8]

During the production of military ammunition, at least in earlier years, little thought was given to its possible disposal or dismantling later and the health risks this entails. The aim of the study was therefore to investigate the current exposure to DNT and TNT during the disposal of military waste and to clarify whether health risks can be observed at this level of exposure.

MATERIALS AND METHODS

A cross sectional study was carried out with 82 employees of a mechanical plant in Saxony, Germany. In this plant primarily military waste containing TNT and occasionally also DNT (for example, armour piercing shells and artillery shells, hand grenades, and bomblets from scatter bombs) had been dismantled since the beginning of the 1990s. The ammunition was opened either manually or by machine (for example, lathe, drill, press) and disassembled into single components. There were exhaust systems at some workplaces. Before the reunification of Germany arms were also produced at this factory. At that time exposure of the employees to DNT and/or TNT did not occur.

The study group (table 1) was divided in two groups by the biological monitoring results (less than analytical detection limit = non-exposed; greater than analytical detection limit = exposed). According to the results of biological monitoring at the time of the examination, 47 exposed persons were employed continuously at different worksites with the disposal of military waste, 11 discontinuously, and five were not involved in the disposal work. Twelve of 19 non-exposed persons were not or only sometimes employed with the disposal of military waste, and four were employed regularly in this worksite, but had control functions.

**Substances for which in vitro or animal studies have yielded evidence of carcinogenic effects that is not sufficient for classification of the substance in one of the other categories.

The investigation included a standardised questionnaire with closed questions on personal medical history, a general physical examination, extensive clinical laboratory tests (for example, methaemoglobin, complete blood count, differential blood count, glucose-6-phosphate dehydrogenase, liver function diagnosis, urine analysis) and biological monitoring of exposure. The analysis of methaemoglobin was performed on the day of sampling by a regional clinical laboratory. For the other analyses the biological samples were stored at −20°C and transported to the clinical and occupational toxicological laboratories of the institute in Erlangen. To quantify the internal exposure, 2,6-DNT, 2,4-DNT, and its main metabolite 2,4-DNBA, as well as TNT and its main metabolites 2-ADNT and 4-ADNT were determined in urine specimens of all study subjects using gas chromatographic/mass spectrometric procedures. For further details of the analytical methods see Angerer and Weismantel[2] and Bader and colleagues.[15] Ambient air analyses were performed by the measurement service of the Employment Accident Insurance Fund of the Chemical Industry. The sampling of ambient air was carried out using personal air samplers and stationary pumps. The analytes (TNT and DNT) were adsorbed onto Tenax tubes and determined after extraction using gas chromatography/mass spectrometry. All investigations were carried out "blind"—that is, the examiner had no knowledge of the particular exposure situation of the person being examined.

Persons were divided by the results of biological monitoring into two groups. If TNT and/or DNT and/or their metabolites were above the analytical detection limit, the persons were defined as "exposed"; if the biological monitoring result was below the analytical detection limit, the persons were defined as "non-exposed". After using the Kolomogorov-Smirnov test, Student's *t* test was applied in case of a normal distribution. In case of a non-normal distribution, the Mann-Whitney test was applied. Differences in frequencies were analysed using the χ^2 test. All tests were calculated two sided and the probability of error set at ≤ 0.05. The correlation coefficient according to Pearson was calculated to determine the correlation between the TNT metabolites.

RESULTS

The exposed persons were employed for a median period of 59 months in the dismantling of military waste (table 1). As the activities varied according to the amount of ammunition to be disposed and of the type and number of arms, a more detailed retrospective differentiation of the exposure situation is not possible. Personal body protection was worn sporadically by only a few study subjects and a classification in this respect was not reasonable.

The values from ambient monitoring showed increased concentrations in the air at some workplaces and in individual work areas, in particular for 2,4,6-TNT (table 2). For the 43 air analyses carried out during the cross sectional study the current German MAK value of 100 µg 2,4,6-TNT/m^3 or 0.011 ppm was exceeded in 13 cases. The highest ambient air concentration was measured during the opening of bomblets from scatter bombs (3250 µg 2,4,6-TNT /m^3). The ambient air concentrations of 2,4-DNT were much lower than the TNT concentrations and reached a maximum of 20 µg/m^3.

The results of the biological monitoring are in line with those from the ambient monitoring (table 3, fig 1). The concentrations of the main metabolites of TNT, 2-ADNT and 4-ADNT, were significantly higher in the group of the regularly exposed workers than in the occasionally or non-exposed group ($p < 0.05$). There was also a highly significant correlation ($r^2 = 0.9491$, $p < 0.001$, $n = 82$) between the urinary concentrations of the two metabolites (fig 2). The regression function was calculated to be:

$$y = 0.23 \times x - 11.41$$

Table 2 Levels of 2,4-DNT and 2,4,6-TNT found in the air in various work areas

Work area	Type of analysis		Measured value or range	
	Stationary air sampling	Personal air sampling	2,4-DNT (μg/m³)	2,4,6-TNT (μg/m³)
Bending hand grenades (work rate approx. 160/h)		x	<0.1–1.0	99–690
Pressing bomblets (work rate: approx. 110/h)		x	0.4–2.5	88–560
Pressing bomblets (work rate: approx. 110/h) (sampling: 2 hours; sample volume: 60 l)	x		<0.5–3.2	22–158
Separating tablets		x	0.2–2.3	54–305
Separating tablets (sampling: 2 hours; sample volume: 60 l)	x		3.7	315
Opening bomblets		x	<0.1–1.3	52–118
Opening bomblets (sampling: 2 hours; sample volume: 60 l)	x		11	3250
Water treatment, removing agglomerate	x		<0.1–4.2	0.4–15
Water treatment, removing agglomerate		x	<0.1–20	1.7–46
Sucking out		x	4.4	24
Sucking out (sampling: 2 hours; sample volume: 60 l)	x		4.4	24
Selection		x	<0.1	3.8–7.5
Selection (sampling: 2 hours; sample volume: 60 l)	x		<0.5	11
Emptying and piercing		x	<0.1	9.5–26
Emptying and piercing (sampling: 2 hours; sample volume: 60 l)	x		<0.5	2.2–25
Hand grenades (intermediate storage) (sampling: 2 hours; sample volume: 60 l)	x		1.0	555
Rest room	x		<0.5	<0.1
Offices (sampling: 2 hours; sample volume: 60 l)	x		<0.5	0.17

Table 3 DNT and TNT and its main metabolites found in urine during biomonitoring

	Concentration in urine (μg/l)		
	No exposure (n=12)	Occasional exposure (n=19)	Regular exposure (n=51)
2,4,6-trinitrotoluene			
Median	<DL	<DL	<DL
Range	<DL–<DL	<DL–<DL	<DL–5.0
n >DL	n=0	n=0	n=6
2-amino-4,6-dinitrotoluene			
Median	<DL	<DL	23.0
Range	<DL–<DL	<DL–<DL	<DL–1464.0
n >DL	n=0	n=0	n=35
4-amino-2,6-dinitrotoluene			
Median	<DL	<DL	231.0
Range	<DL–<DL	<DL–44.0	<DL–6693.0
n >DL	n=0	n=9	n=47
2,4-dinitrotoluene			
Median	<DL	<DL	<DL
Range	<DL–<DL	<DL–<DL	<DL–<2.1
n >DL	n=0	n=0	n=1
2,4-dinitrobenzoic acid			
Median	<DL	<DL	2.1
Range	<DL–3.78	<DL–35.0	<DL–95.0
n >DL	n=3	n=7	n=30
2,6-dinitrotoluene			
Median	<DL	<DL	<DL
Range	<DL–<DL	<DL–<DL	<DL–3.6
n >DL	n=0	n=0	n=5

DL, analytical detection limit.

(y = urinary concentration of 2-ADNT in μg/l and x = urinary concentration of 4-ADNT in μg/l). The main metabolite of DNT in urine, 2,4-DNBA, also reflects the external exposure but the median concentration following regular exposure (2.1 μg/l) was about a factor of hundred lower than the median of 4-ADNT (231 μg/l). This relation corresponds to the concentration ratio of their precursors, TNT and DNT in air.

In their medical history the test persons described the workplace related complaints listed in table 4. Exposed persons reported more frequently bitter taste, burning eyes, and discoloration of the skin or the hair than non-exposed persons.

The clinical laboratory examinations revealed findings outside the normal range (table 5), but no relation to the exposure could be found on a group basis. Besides lactate dehydrogenase (LDH), for all parameters no statistically significant differences were found between the two subgroups. Examination of the study subject with the highest internal TNT exposure (TNT = 5.0 μg/l; 2-ADNT = 6693.0 μg/l; 4-ADNT = 1464.0 μg/l; 2,4-DNT and 2,6-DNT below detection limit; 2,4-DNBA = 95.0 μg/l), who was regularly employed in the dismantling of arms for 59 months, revealed a decreased erythrocyte count (4.44×10^6/mm³) and slightly increased liver values (SGOT = 23 U/l; SGPT = 27 U/l; γ glutamyltransferase

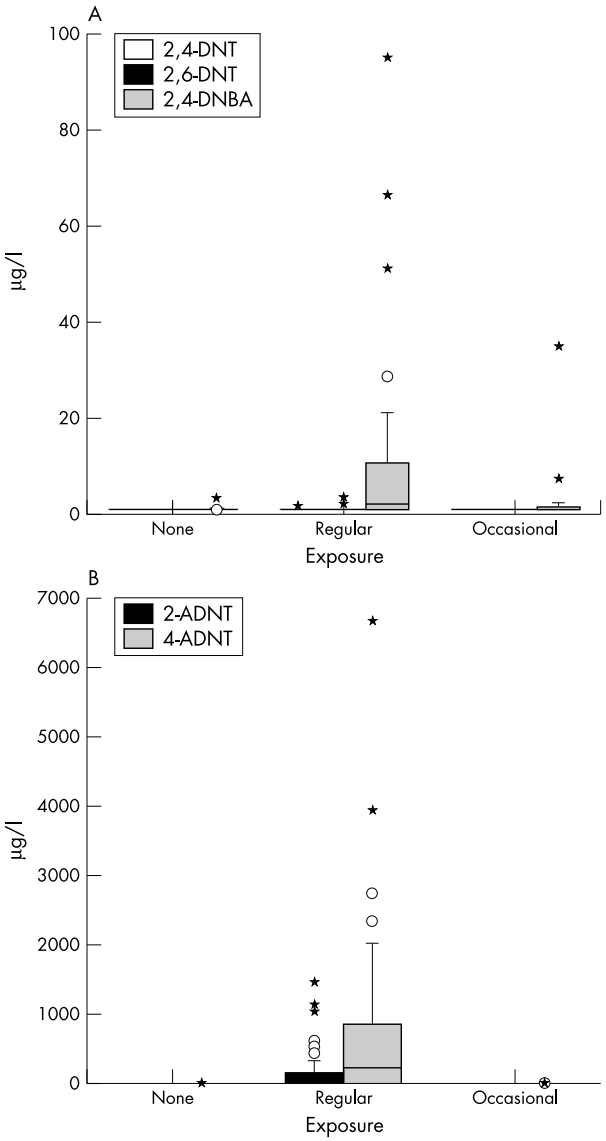

Figure 1 Concentrations of the main metabolites of TNT (A), and 2-ADNT and 4-ADNT (B) in the group of the regularly exposed workers compared to occasionally or non-exposed workers.

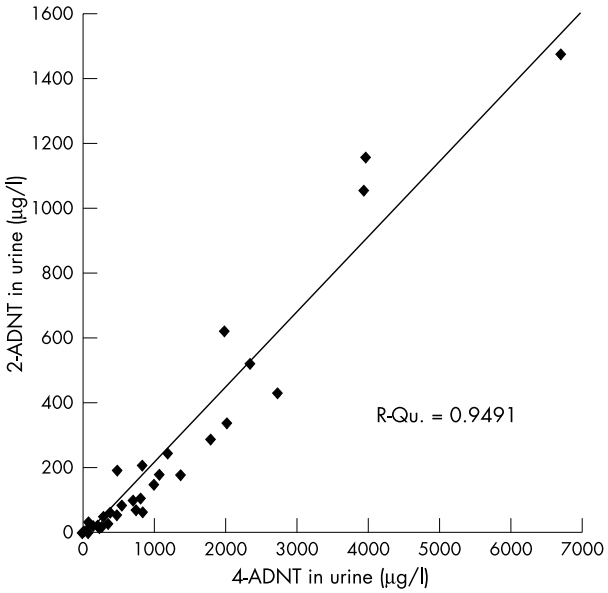

Figure 2 Correlation between the concentration of 2-ADNT and 4-ADNT in urine.

Table 4 Acute and chronic (*) symptoms during occupational activity in the factory investigated (several answers possible)

Complaints	Urinary TNT, DNT, and metabolite concentration <DL (n=19)	>DL (n=63)	p†
Bitter taste	9	48	**0.017**
Drowsiness	2	15	0.211
Headache	1	15	0.074
Burning eyes	0	15	**0.019**
Discoloration of skin/hair*	1	18	**0.035**
Stomach ache	1	7	0.451
Nausea	1	5	0.695
Coughing	2	8	0.800
Running nose	–	2	0.432
Nose bleeds	1	–	0.067

DL, analytical detection limit.
†Significant p values in bold.

= 73 U/l) with a normal cholinesterase level (7025 U/l). The worker stated in his personal medical history that he regularly consumed alcohol at a level of 90 g/day. Taking non-occupational factors into consideration, there was no clear evidence of occupational influences on the results of laboratory tests for the other persons with high internal exposure.

DISCUSSION

With the choice of a cross sectional study, approximately 90% of the workers in the selected factory currently exposed to TNT and/or DNT could be investigated. Selection effects could, however, not be completely excluded as there were insufficient data available on persons who were exposed to TNT and DNT before the investigation and had left the factory.

Ambient monitoring and biomonitoring show that during the dismantling of military waste exposure to hazardous substances can occur of a similarly high level as during the production of ammunition and explosives.[3 10 16–18] Persons were included in the study with relatively high exposures to TNT in some cases. The relatively low DNT values compared to the TNT exposure is explained by the fact that in this particular plant, mainly TNT containing military waste was disposed of; exposure to DNT only occurred occasionally.

The results of ambient monitoring and biomonitoring indicate inadequate measures to minimise TNT and DNT exposure at the individual workplaces. As air analyses were not carried out at all workplaces and most of the study subjects worked at different places in the course of the workshift, correlations between the external and the individual internal exposure were not evaluated.

Taking into account the fact that DNT and TNT are readily absorbed via the skin, and that the use of personal body protection at workplaces with increased exposure to hazardous substances is often neglected by the workers, biological monitoring should be preferred to ambient monitoring to determine the exposure to DNT and TNT and its main metabolites. The linear correlation found in this study between 2-ADNT and 4-ADNT in urine was very similar to a regression function observed in a pilot study under higher TNT exposure which we performed earlier.[15] Therefore the analytical method selected seems to be well suited for the occupational-medical surveillance of persons exposed to TNT.

Table 5 Results of the clinical laboratory examinations

	Urinary TNT, DNT, and metabolite concentration					
	<DL, n=19		>DL, n=63			
Parameter	Median	Range	Median	Range	p*	Normal range
Leucocytes (1000/mm³)	5.5	4.8–9.1	6.6	3.5–15.8	0.094	4.5–12.0
Erythrocytes (10^6/mm³)						
Men	5.12	4.78–5.95	5.05	4.45–5.97	0.229	4.5–5.4
Women	4.90	4.56–5.00	4.54	4.19–5.07	0.180	3.8–5.0
Haemoglobin (mmol/l)						
Men	9.9	9.3–10.5	9.9	8.4–11.3	0.530	8.7–11.2
Women	9.6	8.7–10.0	9.1	8.3–9.7	0.143	7.4–9.9
Haematocrit						
Men	0.464	0.431–0.553	0.457	0.389–0.540	0.624	0.40–0.54
Women	0.442	0.415–0.468	0.428	0.381–0.456	0.196	0.37–0.47
MCV (fl)						
Men	90.3	86.3–93.2	91.5	84.9–99.5	0.124	85–98
Women	92.5	84.2–95.5	90.0	87.2–97.0	0.852	84–98
MCH (fmol)	1.97	1.73–2.07	1.95	1.77–2.17	0.348	1.5–2.05
MCHC (mmol/l)	21.8	18.6–22.3	21.6	20.8–22.3	0.062	19.5–22.1
Methaemoglobin (mmol/mol Hb)						
Smokers	2	1–4	2	0–5	0.864	10–25
Ex-smokers and non-smokers	2	0–3	2	0–8	0.360	2–10
Glucose-6-phosphate dehydrogenase (mU/10^9 erythrocytes)	134	106–156	132	108–178	0.606	75–190
Thrombocytes (1000/mm³)	215	145–303	232	73–332	0.837	150–450
SGOT (U/l)						
Men	14	1–34	14	7–32	0.911	<18
Women	13	10–17	11	8–18	0.631	<15
SGPT (U/l)						
Men	18	9–42	15	6–53	0.413	<22
Women	17	8–27	13	4–35	0.338	<17
γ glutamyltransferase (U/l)						
Men	24	7–259	24	4–213	0.490	<28
Women	13	8–26	15	6–107	1.000	<18
LDH (U/l)	157	128–256	176	116–298	**0.044**	120–240
GLDH (U/l)						
Men	4.9	0.7–16.7	3.0	0.9–22.0	0.180	<4.0
Women	2.7	1.0–6.0	1.8	0.6–6.9	0.425	<3.0
Cholinesterase (U/l)	7033	3945–9341	6435	1916–9177	0.102	3500–8500
Creatinine (serum) (mg/dl)						
Men	0.97	0.79–1.28	0.99	0.75–1.34	0.322	<1.3
Women	0.89	0.78–0.95	0.89	0.77–1.11	0.564	<1.1
Apo-A_1 (g/l)	1.53	0.96–2.05	1.52	0.93–2.59	0.497	1.02–2.15
Apo-B (g/l)						
Men	1.36	0.93–2.40	1.29	0.67–2.23	0.263	0.55–1.65
Women	1.23	0.62–2.34	1.19	0.52–1.46	0.521	0.60–1.44

DL, analytical detection limit.
*Significant p values in bold.

With regard to the strength of correlation between the two TNT metabolites, the determination of one of these metabolites seems to be sufficient for occupational-medical health surveillance. In view of the higher concentrations observed for 4-ADNT, we recommend this parameter for biological monitoring of occupational exposure to TNT. 2,4-DNBA turned out to be a specific and sensitive parameter for internal exposure to DNT. This metabolite was found in 40 of 82 urine samples (4-ADNT: 56/82), though DNT concentrations in most working areas were much lower.

The anamnestic data of the study subjects on workplace related complaints, such as a bitter taste, burning eyes, and discoloration of skin and hair correlate well with data in the literature,[3 9] and also indicate relevant uptake of the hazardous substance.

Glucose-6-phosphate dehydrogenase deficiency was not detected in any of the workers, so TNT induced haemolytic anaemia, as described in the literature,[10 11] was not observed. Furthermore, for the other clinical laboratory parameters measured, no substance specific effects were found when the groups were compared, taking into account the influence of the different proportions of the genders in the two groups and non-occupational risk factors. Deviations from reference values found in individuals in all subgroups followed no pattern of distribution which could be clearly attributed to the occupational exposure. The differences in the LDH concentrations between the two groups might be due to differing physical exercise in the two groups. In contrast to the literature data we could not detect dose-response relations in our study despite the high exposure to nitroaromatics.[3 4 9] A possible reason for this is the relatively short exposure period of the workers (occasional exposure: 25 to 59 months; regular exposure: 7 to 59 months). It must also be pointed out that the working characteristics and exposure situation in the plant were dependent on the changing types of ammunition and special requirements. Therefore, the concentrations of hazardous substances measured at the time of the investigation are not necessarily representative of the whole exposure period. Selection effects are also possible as persons with substance related damage to the health may already have left the factory. This aspect could not be adequately considered because of a significant reduction of the workforce in the years previous to the cross sectional study. It could also not be excluded that the methods used were not sufficiently sensitive to detect early stages and bridging symptoms of DNT and TNT induced health disturbances.

To summarise, it can be concluded that the results of the investigation revealed levels of exposure to TNT and DNT during the disposal of military waste of occupational-medical

relevance. The disturbances in wellbeing described by the workers point out the necessity for an improvement of protective measures and adequate occupational-medical surveillance during the disposal of military waste. Further studies are necessary to exclude the possible occurrence of long term effects.

ACKNOWLEDGEMENT

We are grateful to the "Berufsgenossenschaft der chemischen Industrie", in particular Dr Krommes and Dipl.-Ing. W Kurth, for supporting the investigation and carrying out the air analyses.

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This paper is dedicated to Prof. Dr. J Angerer's 60th birthday

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ECHO

Safety records confirm the risks of professional horse racing

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A review of injuries in professional horse racing in Great Britain and the Irish Republic has found that racing tops even mountaineering for fatal injuries. The review covers 1992 to 2000 and provides the first accurate analysis of racing injuries.

Minor soft tissue injuries were the commonest; next were more serious injuries such as fractures—mostly to the arms or collarbone. Concussion was common (1.8–7.4/100 falls in jump or flat racing, respectively), especially in flat racing because of its greater speed and tendency for the horses to bunch and for fallen jockeys to be kicked. More serious head injuries occurred only three times.

Despite the apparent dangers deaths during the review period were rare, just two in Great Britain, both from internal injuries. Sixteen jockeys sustained injuries in Great Britain during 1996–2000 that ended their career.

These data are available thanks to compulsory safety measures, including strict monitoring introduced in 1992 by racing's regulatory bodies in Great Britain and the Irish Republic. Falls and injuries must be recorded in a jockey's medical record book by one of the doctors solely responsible for jockeys at each race meeting; a written record is returned to the chief medical advisor of the regulatory body.

The review was based on searches of Medline and Sport Discus 1975–2001, hand searches, and contacts with experts and sports organisations worldwide.

Injuries to professional jockeys have not been much reported—despite racing's high profile as a spectator sport—and epidemiological data are particularly scarce.

▲ *British Journal of Sports Medicine* 2002;**36**:403–409.