Hemoglobin adducts in workers exposed to nitrotoluenes

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Nitrotoluenes are important intermediates in the chemical industry. 2,6-Dinitrotoluene (26DNT), 2,4-dinitrotoluene (24DNT) and 2-nitrotoluene are carcinogenic in animals and possibly carcinogenic in humans. It is therefore important to develop methods to biomonitor workers exposed to such chemicals. Hemoglobin (Hb) adducts of nitroarenes are established markers of the biological effective dose. We developed a method to measure Hb adducts in biological samples. Hb adducts were measured in rats after a single exposure (0.5 mmol/kg) of 24DNT, 26DNT, 2,4-toluenediamine (24TDA) and 26TDA. Hydrolysis of Hb from rats dosed with 24DNT yields, 4-amino-2-nitrotoluene (4A2NT) (16.3 nmol/g Hb), 24TDA (4.3 nmol/g Hb) and 4-acetylaminoo-2-aminotoluene (4AA2AT) (0.51 nmol/g Hb). Hydrolysis of Hb from rats dosed with 26DNT yields three amines, 2-amino-6-nitrotoluene (2A6NT) (2.5 nmol/g Hb), 26TDA (1.2 nmol/g Hb) and 2-acetylamino-6-aminotoluene (2AA6AT) (0.17 nmol/g Hb). A similar Hb adduct pattern was found in Chinese workers exposed to nitrotoluenes. With respect to 24DNT, 4A2NT was the predominant adduct, and the amount was ~24-fold higher than 24TDA. With respect to 26DNT, 2A6NT was the predominant adduct, and the amount was ~20-fold higher than 26TDA. With respect to the mononitrotoluenes, the Hb adduct of 2NT was present in the highest concentrations. Each worker was examined for adverse health effects related to exposure to DNT. The health effects were compared with the Hb adduct levels using logistic regression analysis. The odds of suffering from inertia were 3.2 times higher [95% confidence interval (CI) = 1.8–5.8] when the level of 4A2NT Hb adducts increased by one log-unit. Similar odd ratios were observed with somnolence (3.1, CI = 1.4–6.9), nausea (2.4, CI = 1.3–4.3) and dizziness (5.5, CI = 1.3–24.2). These results inferred that quantification of DNT–Hb adducts provided an effective biomarker of toxicity and could be used to estimate the risk associated with a particular exposure to DNT.

Introduction

Dinitrotoluenes (DNT) and mononitrotoluenes (NT) are important intermediates in the chemical industry. There is clear evidence that 2-nitrotoluene (2NT) caused cancer in rats and mice (1). In contrast 4-nitrotoluene (4NT) caused equivocal carcinogenic effects in rats and mice (2,3). 2,6-Dinitrotoluene (26DNT) and 2,4-dinitrotoluene (24DNT) have been classified as carcinogenic in animals and as possibly carcinogenic in humans (4). In two rat studies technical dinitrotoluene (t-DNT)—a mixture of 24DNT and 26DNT—induced hepatocellular carcinomas (5,6). However, conclusions drawn from the isomer-specific carcinogenicity study by Leonard et al. (6) and tumor-initiation/promotion assays by Popp and Leonard (7) suggest that 26DNT rather than 24DNT is the primary hepatocarcinogen in t-DNT. An excess of hepatobiliary cancer was found among munition workers exposed to dinitrotoluenes (8) and Brüning et al. (9) found urethal tumor cases predominantly confined to miners highly exposed to DNT. It is therefore important to develop methods to monitor exposed populations.

DNT and NT are metabolized by the reduction of the nitro group(s) and/or oxidation of the methyl group (reviewed in ref. 10). One or both of the nitro groups may be reduced to the corresponding aminonitrotoluenes, toluidinediamines or aminotoluenes, while the methyl group is oxidized to a benzyl alcohol or benzoic acid. Aminonitrotoluenes, toluidinediamines and aminotoluenes can be further N-oxidized to yield N-hydroxyarylamines. The N-hydroxyarylamines and benzylalcohols may undergo further conjugation with sulfate, glucuronide or acetyl. The secondary products of N-oxidation or methyl-oxidation are responsible for the genotoxic and cytotoxic effects of these compounds. The initiation of chemical carcinogenesis generally involves the covalent binding of xenobiotics, or their reactive metabolites, with nucleophilic DNA centres (11,12). Hemoglobin (Hb) in blood erythrocytes is a molecular target for reactive electrophilic species, and thus has been used as a surrogate dosimeter to measure the proportion of exposure, which attacks nucleophilic targets such as DNA (13,14, reviewed in ref. 15). Therefore, Hb adducts are an excellent tool to biomonitor exposed workers. Hb adducts result from N-hydroxyarylamines, which are oxidized to nitrosoamines in the erythrocytes and then form sulfiamide adducts with cysteine residues in Hb (Figure 1). 2NT forms Hb and DNA adducts in rats (16). DNA adducts of DNT and TDA were investigated by Froines’ group (17–19). 24DNT, 26DNT and 2,4-toluenediamine (24TDA) bind to liver DNA but 26TDA does not. Hb adducts in rats have been investigated by Froines’ group (19–21) and Neumann’s group (22) whereby

Abbreviations: 2AA4AT, 2-acetylamino-4-aminotoluene; 4AA2AT, 4-acetylamino-2-aminotoluene; 2AA6AT, 2-acetylamino-6-aminotoluene; 2A4NT, 2-amino-4-nitrotoluene; 4AA2NT, 4-amino-2-nitrotoluene; 2A6NT, 2-amino-6-nitrotoluene; d, deuterated; DNT, dinitrotoluenes; 26DNT, 2,6-dinitrotoluene; 24DNT, 2,4-dinitrotoluene; EI, electron impact ionization; Hb, hemoglobin; 2MA, 2-methylalanine; 3MA, 3-methylalanine; 4MA, 4-methylalanine; NCI, negative chemical ionization; NT, mononitrotoluenes; 2NT, 2-nitrotoluene; 4NT, 4-nitrotoluene; OR, odds ratio; PFFPA, pentafluoropropionic anhydride; t-DNT, technical dinitrotoluene; 24TDA, 2,4-toluenediamine; 26TDA, 2,6-toluenediamine; TNT, trinitrotoluene.

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Hb adducts were found in rats dosed orally with 24TDA, 26TDA, 26DNT and 24DNT. The results obtained by the two groups were very different. Therefore, for the present work the experiments with rats were repeated. The Hb adduct profile in rats will be compared with the adduct profile present in Hb from a collective of Chinese workers involved in the production of trinitrotoluene (TNT). Blood samples, work place description, air measurements and medical examinations were collected from Chinese workers and non-matching controls. Hb adducts were determined for all subjects. The Hb adduct levels were compared with the other measured parameters in order to find dose-related effects of nitrotoluene exposures.

**Materials and methods**

**Materials**

2-Amino-4-nitrotoluene (99%) (2A4NT), 4-amino-2-nitrotoluene (99%) (4A2NT), 2-amino-6-nitrotoluene (99%) (2A6NT), 2-methylalanine (99.9%) (2MA), 3-methylalanine (99%) (3MA), 4-methylalanine (99.9%) (4MA), 2,4-toluenediamidine (99%) (24TDA) and 2,6-toluenediamidine (99%) (26TDA) were obtained by Fluka. 2-Acetylamino-4-aminotoluene (2AA4AT), 4-acetylamino-2-aminotoluene (4AA2AT) and 2-acetylamino-6-aminotoluene (2AA6AT) were synthesized according to the procedures of Sabbioni and Beyerbach (25,26). d6-4AA2AT, d6-4A2NT, d6-2A4NT, d6-2A6NT and d6-2AA6AT were synthesized following the procedures for the corresponding non-deuterated compounds 4AA2AT (23,24), 4A2NT (27), 2A4NT (27) and 2A6NT (27), respectively. The compounds were characterized by GC-MS. Electron impact ionization (EI-MS) (70eV, m/z (%): d6-2A4NT: 159 (9%), 158 (M+), 90, 157 (23), 156 (3), 140 (22), 112 (87), 100 (29), 85 (42), 84 (87), 83 (1), 81 (100), 80 (50), 66 (21), 54 (37), d6-2A6NT: 159 (5%), 158 (M+), 44, 157 (10), 140 (41), 112 (100), 110 (35), 85 (47), 84 (55), 82 (42), 81 (73), 80 (31), 54 (32), d6-2A6NT: 159 (8%), 158 (M+), 70, 157 (5), 140 (49), 112 (100), 85 (69), 84 (94), 83 (22), 82 (52), 81 (87), 80 (37), 69 (22), 66 (18), 57 (34), 56 (33), 54 (48). d6-4AA2AT: 170 (M+), 25, 169 (31), 168 (46), 167 (22), 127 (53), 126 (90), 125 (100), 124 (59), 108 (24), 98 (24), 81 (26), 53 (24).

**Methods**

**Questionnaire and medical examination.** The study was performed in accordance with the principles embodied in the declaration of Helsinki (www.wma.net/e/policy/b3.htm). Informed consent was obtained from each worker. Blood collection, medical examination and questionnaire were all performed in the same week. Each participant was interviewed, using the questionnaire, about their general status, exposure history, smoking and alcohol consuming habits, previous medical record and present symptoms. The Medical Department of the Chinese Academy of Preventive Medicine performed the following examinations: (i) physical examinations: blood pressure, cardiovascular system, nervous system and heart rate. (ii) Routine blood and urine tests, liver function test: glumatic pyruvic transaminase, alkaline phosphatase, total protein, albumin, total bilirubin. (iii) Electrocardiogram. (iv) Ultrasonic type B examination for liver and spleen. (v) Serological assays of hepatitis B antigens and antibodies were conducted, because hepatitis B is common in China, and liver damage can also be caused by some of the nitroarenes.

**Collection of blood samples.** Blood samples were collected from employees manufacturing dinitrotoluenes and TNT in a factory situated in Liaoning (Liaoning Province, China). In total, 160 blood samples were collected from the DNT factory workers. Of these, 99 were from exposed workers and 61 were from non-matched, non-exposed controls working in the same factory. Blood samples were collected in EDTA tubes. The erythrocytes were separated from the plasma by centrifugation. The erythrocytes were washed three times with an equal amount of 0.9% saline buffer. The erythrocytes were stored below −20°C and shipped to Europe on dry ice. The erythrocytes were lysed by adding 4 vol of water. Cell debris was removed by centrifugation. Hb was precipitated with ethanol from the lysed erythrocytes. The precipitate was washed with ethanol–water (8:2), ethanol (2 times), ethanol–diethyl ether (3:1) and diethyl ether. The washed Hb was dried in a desiccator.

**Hb hydrolysis.** Hb (100 mg human Hb or 20 mg rat Hb) was dissolved in 0.1 M NaOH (2 ml) by vortex mixing in glass tubes (100 × 22 mm). The hexane solution (10 µl) with the internal standards (1 ng/10 µl of d6-2MA, d6-3MA, d6-4MA, d6-24TDA, d6-26TDA, d6-24DNT and d6-26DNT and 2 ng/10 µl d6-4AA2AT) was added to the basic Hb solution (pH >12). After 1 h in a shaking bath at room temperature the Hb was extracted with CH2Cl2 (3 ml). The mixture was vortex mixed for 1 min then separated by centrifugation at 2000 g for 10 min. The samples were frozen in liquid nitrogen, then thawed at room temperature to facilitate phase separation. The organic layer was dried through a pipette containing anhydrous Na2SO4 (1 g) and washed with CH2Cl2 (1.5 ml). The dried organic phase was collected in a graduated tapered tube.
Table I. Retention time and major EI- and NCI-mass fragments determined from full scan spectra m/z 50-350 of the PFPA derivatives of arylamines

<table>
<thead>
<tr>
<th>PFPA derivatized arylamine</th>
<th>Elution time (min)</th>
<th>Major mass fragments for GC-MS-SIM analysis</th>
<th>Deuterated internal standard</th>
<th>NCI ions m/z (%)</th>
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</thead>
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<tr>
<td>2MA</td>
<td>3.27</td>
<td>235</td>
<td>d3-2MA</td>
<td>237</td>
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<tr>
<td>3MA</td>
<td>3.37</td>
<td>233</td>
<td>d3-3MA</td>
<td>237</td>
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<tr>
<td>4MA</td>
<td>3.42</td>
<td>233</td>
<td>d3-4MA</td>
<td>237</td>
</tr>
<tr>
<td>26TDA</td>
<td>4.41</td>
<td>394 (100), 374 (10)</td>
<td>d4-26TDA</td>
<td>399 (100), 399 (97)</td>
</tr>
<tr>
<td>24TDA</td>
<td>4.50</td>
<td>394 (100), 374 (42)</td>
<td>d4-24TDA</td>
<td>399 (19), 399 (100)</td>
</tr>
<tr>
<td>2A6NT</td>
<td>4.33</td>
<td>298 (5), 278 (100)</td>
<td>d4-2A6NT</td>
<td>284</td>
</tr>
<tr>
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<td>298 (14), 278 (100)</td>
<td>d4-4A2NT</td>
<td>284</td>
</tr>
<tr>
<td>2A4NT</td>
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<td>298 (5), 278 (100)</td>
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<td>284</td>
</tr>
<tr>
<td>2AA2AT</td>
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<td>290 (100), 289 (16)</td>
<td>d4-2AA2AT</td>
<td>296 (75), 295 (100)</td>
</tr>
<tr>
<td>4AA2AT</td>
<td>5.97</td>
<td>290 (100), 289 (22)</td>
<td>d4-4AA2AT</td>
<td>296 (75), 295 (100)</td>
</tr>
</tbody>
</table>

The presented ions were chosen for single ion monitoring of arylamines in Hb extracts of workers exposed to mixed nitrotoluenes. The arylamines were separated on a silica fused capillary column (Phenomenex ZBS, 15 m × 0.25 mm i.d., 0.5-μm film thickness).

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Hemoglobin adducts in workers exposed to nitrotoluenes

The exposure and health effects of Chinese working in a TNT factory were investigated. The production of TNT involves the exposure to high levels of the intermediate products NT and DNT, which are far more volatile than TNT. In the Chinese factory, the synthesis of TNT was performed by continual batch nitration of NT, then DNT, with sulphuric acid and nitric acid. The workers were grouped according to their job description and work location as follows: group leader, NT-tank, DNT-tank, laboratory of chemical analyses, transportation of TNT to packaging, packaging, control room, disposal of waste acid, disposal of waste H₂O and non-exposed control workers. All participants filled out a questionnaire and their health status was checked (see Materials and methods). Blood samples were collected from 99 exposed workers and from 61 non-matched, non-exposed controls working in the same factory.

Results and discussion

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Determination of Hb adducts in Chinese workers exposed to mono- and dinitrotoluenes

Exposure to mixed nitrotoluenes in workers was determined by measurement of the level of arylamine-cleavage products.
released from Hb following mild base hydrolysis. The full scan mass spectra m/z 50–500 of PFPA derivatized standards, 2MA, 3MA, 4MA, 26TDA, 24TDA, 2A4NT, 4A2NT, 2A6NT, 2AA4AT, 4AA2AT and 2AA6AT were acquired in both NCI and EI modes. For analysis of low levels of the above amines in human Hb samples, increased sensitivity was achieved by selection of the most abundant characteristic fragments, determined in the full scan spectra of authentic standards. These selected ions were acquired in single ion monitoring and have been presented in Table I.

The PFPA derivatized arylamines were identified by their characteristic mass fragments in the NCI mode, and by their retention times with respect to authentic deuterated internal standard (Table I). Single ion chromatograms, acquired in the NCI mode, have been presented in Figure 2. The presented chromatograms were from CH₂Cl₂ extracts of the Hb hydrolyzates from an exposed worker and a control worker. The methylanilines 2MA, 3MA and 4MA were observed at 3.27, 3.37 and 3.42 min, respectively. The toluenediamines, 26TDA and 24TDA, were observed at 4.41 and 4.50 min, respectively. The aminonitrotoluenes, 2A6NT and 4A2NT, were observed at 4.33 and 4.66 min and the acetylated adduct 4AA2AT at 5.97 min.

For further characterization of the identified arylamine adducts, PFPA-derivatized Hb extracts from a selection of the highest exposed workers were analyzed in the EI mode. As a result of the limits of sensitivity only 4A2NT was observed.

There were a number of arylamine adducts identified in the organic extracts of base hydrolyzed Hb from workers exposed to mixed nitrotoluenes. These included (i) methylanilines, 2MA, 3MA and 4MA, which were indicative of exposure to 2NT, 3NT and 4NT, (ii) the toluenediamines, 26TDA and 24TDA and (iii) the aminonitrotoluenes, 2A6NT and 4A2NT, which were indicative of exposure to 24DNT and 26DNT. Only one acetylated arylamine, 4AA2AT, was observed in the extracts of Hb hydrolyzates from exposed workers. The aminonitrotoluene, reduced at the ortho position (2A4NT), and the acetylated amines, 2AA4AT and 2AA6AT, were not identified in the single ion chromatograms of exposed workers.

Quantification of arylamines covalently bound to Hb was based upon the analytical procedure described by SABBIONI and BEYERBACH (25, 26). The level of hydrolyzable Hb-arylamine adducts present in each Hb (100 mg) was calculated from a calibration line of known standards spiked into bovine Hb and taken through the assay procedure. The ion abundance of each amine peak, [M-HF]⁺, detected in the single ion chromatogram was related to that of the deuterated internal standard. The ratio was then compared with the abundance ratio determined for the calibration line.

Job-related exposure to mono- and dinitrotoluenes
Hb adducts were found in the exposed workers and in parts of the controls working in the same factory. The box plots, presented in Figure 3 show the median Hb adduct levels of 2MA, 3MA, 4MA, 26TDA, 24TDA, 2A6NT and 4A2NT, with the 10th, 25th, 75th and 90th percentiles presented as vertical boxes and error bars. 2MA, 3MA and 4MA were found in all exposed and factory controls. 4A2NT, 2A6NT, 24TDA and 26TDA were found in 99, 96, 100 and 85% of the exposed workers, respectively. In the factory controls 4A2NT, 2A6NT, 24TDA and 26TDA were found in 99, 96, 100 and 85% of the exposed workers, respectively. In the factory controls 4A2NT, 2A6NT, 24TDA and 26TDA were found in 99, 96, 100 and 85% of the exposed workers, respectively. In the factory controls 4A2NT, 2A6NT, 24TDA and 26TDA were found in 99, 96, 100 and 85% of the exposed workers, respectively. In the factory controls 4A2NT, 2A6NT, 24TDA and 26TDA were found in 99, 96, 100 and 85% of the exposed workers, respectively. In the factory controls 4A2NT, 2A6NT, 24TDA and 26TDA were found in 99, 96, 100 and 85% of the exposed workers, respectively. In the factory controls 4A2NT, 2A6NT, 24TDA and 26TDA were found in 99, 96, 100 and 85% of the exposed workers, respectively. In the factory controls 4A2NT, 2A6NT, 24TDA and 26TDA were found in 99, 96, 100 and 85% of the exposed workers, respectively. In the factory controls 4A2NT, 2A6NT, 24TDA and 26TDA were found in 99, 96, 100 and 85% of the exposed workers, respectively. In the factory controls 4A2NT, 2A6NT, 24TDA and 26TDA were found in 99, 96, 100 and 85% of the exposed workers, respectively. In the factory controls 4A2NT, 2A6NT, 24TDA and 26TDA were found in 99, 96, 100 and 85% of the exposed workers, respectively. In the factory controls 4A2NT, 2A6NT, 24TDA and 26TDA were found in 99, 96, 100 and 85% of the exposed workers, respectively. In the factory controls 4A2NT, 2A6NT, 24TDA and 26TDA were found in 99, 96, 100 and 85% of the exposed workers, respectively.
24TDA and 26TDA were present in 62, 31, 64 and 62% of the workers. 4AA2AT was found in 20% of the exposed workers. This is probably due to the limit of determination, which is 40 times higher compared with the other compounds, and to the chromatographic properties of 4AA2AT, which yields broad peaks.

The mean and median adduct levels decrease in the following order: 4A2NT, 2A6NT, 2MA, 24TDA, 4MA, 26TDA and 3MA (Figure 3). For the cases where 4AA2AT was found the mean levels were similar to 24TDA. The groups of workers were compared using a one-way ANOVA test of the logarithmically transformed data or using a non-parametric method. The mean of all adducts are significantly higher in the exposed group than in the factory control group using the ANOVA test, except for 3MA. For all adducts the median adduct levels were significantly higher in the exposed workers as determined by the Mann–Whitney test ($P < 0.0001$). It appears that the factory environment is contaminated as we did not find adducts derived from DNT in the controls of our laboratory.

Adducts of 2MA, 3MA and 4MA have been found in the general human population in other studies.

The workers were grouped according to their job description as follows: group leader ($n = 14$), NT-tank ($n = 15$), DNT-tank ($n = 7$), TNT-tank ($n = 19$), analysis laboratory ($n = 18$), transportation of TNT to packaging ($n = 2$), packaging ($n = 7$), control room ($n = 12$), disposal of waste acid ($n = 3$), and control workers ($n = 61$). For workers grouped into waste acid, waste H$_2$O or transportation of TNT, statistical analysis was not performed because the sample number was too low ($n < 5$). In general the adduct levels decrease in the following order among the worker groups: analysis > TNT-tank > NT-tank > DNT-tank > group leaders > control room workers > packing > factory controls. The relative levels of each DNT Hb adduct are closely paralleled between the differently grouped workers, such that high levels of 4A2NT in analysis workers are associated with high levels of 24TDA, 2A6NT or 26TDA in the same analysis workers. The most significant differences among the groups were found for adducts of 2MA, 2A6NT, 4A2NT and 24TDA using the Mann–Whitney test. From 28 possible comparisons between worker groups six, seven, nine and nine comparisons were not significantly different for 24TDA, 4A2NT, 2A6NT and 2MA, respectively. In Figure 4, the adduct levels of 4A2NT are presented as an example. All the differences between the groups are significant ($P < 0.05$) for 4A2NT adducts, except for the following seven comparisons: factory controls versus packers; analysis versus TNT-tank workers; TNT-tank versus DNT-tank workers; TNT-tank versus NT-tank workers; DNT-tank versus NT-tank workers; DNT-tank workers versus group leaders.

**Correlation between different adducts**

The combined level of Hb adducts, in exposed workers, resulting from exposure to 24DNT [mean ± SD (median, 25th and 75th percentiles): 71.0 ± 68.9 pmol/g (51.9, 18.6 and 84.3)] are ~11-fold higher than Hb adduct levels resulting from exposure to 26DNT [6.5 ± 6.5 (4.3, 2.1 and 8.2)], and ~7-fold higher than Hb adduct levels resulting from exposure to NT [10.8 ± 9.8 (9.2, 6.5 and 12.1)]. Among the adducts resulting from DNT exposure, the aminonitrotoluenes are bound to Hb at much higher concentrations than the analogous toluenediamines. With respect to 26DNT, 2A6NT is the predominant adduct, and the amount is ~20-fold higher than 26TDA. With respect to 24DNT, 4A2NT is the predominant adduct, and the amount is ~24-fold higher than 24TDA. With respect to the mononitrotoluenedamines, the Hb adduct of 2NT is present in the highest concentrations. The levels of 2MA–Hb...
are ∼5- and 4-fold higher than 3MA–Hb and 4MA–Hb adducts, respectively.

The adduct levels of the major adducts found in over 96% of the exposed workers were compared using correlation analysis to determine to what extent one amine could predict the internal exposure dose of another amine. The major adducts found in the workers were 2MA, 24TDA, 2A6NT and 4A2NT. Linear regression analysis was performed on the raw Hb adduct data (pmol/g Hb) and on the log-transformed data (log base 10 of Hb data pmol/g Hb). In addition, the data were compared by the Spearman rank test. The statistical significance associated with each correlation has been presented along with the correlation coefficients in Table II. The correlation coefficients obtained from the log-transformed data are almost identical to the correlation coefficients obtained from the Spearman rank test. In contrast the correlation coefficients obtained from the linear regression of the raw data differ substantially in some cases, indicating that outliers skew the analyses. In the following text the correlation coefficients of the log-transformed data are discussed. There is a high correlation between the aminonitrotoluenes, 2A6NT and 4A2NT ($r = 0.94$, Figure 5). The association between these amines and the total level of combined Hb adducts of 24DNT and 26DNT is very strong ($r = 0.96$ for 2A6NT and 0.99 for 4A2NT) and confirms that these amines strongly predict the total level of hydrolyzable Hb adducts of 24DNT and 26DNT. These amines give a good indication of the absorbed dose following exposure to 24DNT and 26DNT in exposed workers. There is a high correlation between the level of 24TDA and 2A6NT ($r = 0.82$) or 4A2NT ($r = 0.87$). For 4A2NT, 2A6NT and 24TDA, correlation coefficients of the log-transformed data are very close to the Spearman rank test, and comparable with the raw data, which confirms that the raw data are not adversely skewed, and no one data point takes too much weight in the regression line.

For adducts of the mononitrotoluenes there is a strong association ($r = 0.97$) between the level of 2MA and the total level of combined methylamines, resulting from hydrolysis of Hb adducts of 2NT, 3NT and 4NT. The association

### Table II. Correlation analysis between Hb adducts found in 96–100% of workers ($n = 99$) exposed to mixed nitrotoluenes

<table>
<thead>
<tr>
<th>Hb adduct (pmol/g Hb)</th>
<th>Correlation coefficient $r$</th>
<th>24TDA</th>
<th>2A6NT</th>
<th>4A2NT</th>
<th>Total NT</th>
<th>Total DNT</th>
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<tr>
<td>2MA</td>
<td>$r$ (ranks)</td>
<td>0.48$^d$</td>
<td>0.44$^d$</td>
<td>0.43$^d$</td>
<td>0.96$^d$</td>
<td>0.43$^d$</td>
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<td></td>
<td>$r$ (log-data)</td>
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<td>0.46$^d$</td>
<td>0.49$^d$</td>
<td>0.97$^d$</td>
<td>0.48$^d$</td>
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<tr>
<td></td>
<td>$r$ (raw-data)</td>
<td>0.09$^f$</td>
<td>0.07$^f$</td>
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<td>0.08$^f$</td>
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<tr>
<td>24TDA</td>
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<tr>
<td>2A6NT</td>
<td></td>
<td>0.93$^d$</td>
<td>0.47$^f$</td>
<td>0.94$^d$</td>
<td>0.93$^d$</td>
<td>0.93$^d$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.94$^d$</td>
<td>0.50$^d$</td>
<td>0.96$^d$</td>
<td>0.93$^d$</td>
<td>0.93$^d$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.92$^d$</td>
<td>0.11$^f$</td>
<td>0.93$^d$</td>
<td>0.92$^d$</td>
<td>0.92$^d$</td>
</tr>
<tr>
<td>4A2NT</td>
<td></td>
<td>0.48$^d$</td>
<td>1.00$^d$</td>
<td>0.99$^d$</td>
<td>0.52$^d$</td>
<td>0.99$^d$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.52$^d$</td>
<td>1.00$^d$</td>
<td>0.99$^d$</td>
<td>0.13$^f$</td>
<td>1.00$^d$</td>
</tr>
</tbody>
</table>

Correlation coefficient $r$ determined from the (i) ranks (Spearman rank test), (ii) logarithmic transformed data (Pearson correlation) and (iii) the raw data (Pearson correlation).

$^a$Sum of 2MA, 3MA and 4MA resulting from exposure to 2NT, 3NT and 4NT.

$^b$Sum of 26TDA, 24TDA, 2A6NT and 4A2NT resulting from exposure to 26DNT and 24DNT.

$^c$The sum of NT and DNT.

The statistical significance of each correlation was given by the $P$ value.

$^d P < 0.0001$, $^e P < 0.05$, $^f P > 0.05$. 

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**Fig. 4.** Box plot showing the median level of 4A2NT determined in Hb from workers exposed to mixed nitrotoluenes. Workers were grouped according to their job description. The 10th, 25th, 75th and 90th percentiles have been presented as vertical boxes with error bars, and outliers have been highlighted as circles. The median levels of 4A2NT were statistically different ($P < 0.05$) between groups according to the Mann–Whitney test, except for the pairs a, b, c, d, e, f, g.

**Fig. 5.** Linear regression analysis was performed on the PFPA derivatized alyramines (pmol/g Hb) detected in hydrolyzed Hb from workers exposed to mixed nitrotoluenes. A regression line was fitted to the correlation between the adducts 2A6NT and 4A2NT ($r = 0.94$).
between 2MA and 3MA or 3MA and 4MA is poor \((r < 0.36)\) but between 2MA and 4MA is moderate \((r = 0.57)\). There is a moderate association \((r < 0.51)\) between the methylanilines, 2MA and 4MA, and the DNT adducts 24TDA, 4A2NT or 2A6NT. There is a poor association \((r < 0.21)\) between 3MA and the DNT adducts. These results indicate that for NT exposure, only 2MA and 4MA are job-related adducts.

In conclusion, 4A2NT is the best marker to monitor workers exposed to DNT: 4A2NT is the major adduct and present in 100% of the workers. 4A2NT correlates with the other major adducts resulting from DNT exposure. For exposure to 2MA and 4MA, and the DNT adducts 24TDA, 4A2NT or 2A6NT, there is a poor association \((r = 0.21)\) between 2MA and 4MA, and a moderate association \((r = 0.36)\) between 2MA and 3MA or 3MA and 4MA. There is a poor association \((r < 0.21)\) between 3MA and the DNT adducts. These results indicate that for NT exposure, only 2MA and 4MA are job-related adducts.

Identification and quantification of Hb adducts in dosed rats

Experiments to identify Hb adducts in rats \((n = 3)\) dosed with 0.5 mmol/kg 24TDA, 26TDA, 24DNT and 26DNT were performed in order to corroborate the Hb adducts identified in the DNT workers. The average level of each arylamine detected in the PFPA-derivatized extracts of base hydrolyzed Hb (three rats per compound analyzed in duplicate) has been presented in Table III. From these results the Hb binding index (HBI) was determined for each cleavage product and also for the total amount of compound bound to Hb.

Hydrolyzable Hb adducts were found for each of the compounds investigated. The same spectrum of adducts was observed in the rat Hb extracts as determined in the Hb extracts from workers exposed to 24DNT and 26DNT.

Hydrolysis of Hb from rats dosed with 24TDA yields two amines, 24TDA and 4A2AT. The levels of 4AA2AT are ~5-fold higher than 24TDA. Hydrolysis of Hb from rats dosed with 24DNT yields three amines, 2A6NT, 24TDA and 2AA6AT. The level of 26TDA is equivalent in rats dosed with 24TDA or 24DNT, where the level of 24TDA is 31-fold higher in the rats administered 24DNT. The level of the acetylated amine, 2AA6AT, found in rats dosed with 26DNT is 5.7-fold lower than 26TDA.

Hb adduct levels are higher in rats dosed with the dinitrotoluenes compared with rats dosed with toluenediamines. Hb adduct levels in rats dosed with 24DNT are higher than adduct levels in rats dosed with 26DNT. In contrast Hb adduct levels in rats dosed with 24TDA are lower than in rats dosed with 26TDA.

Generally, less oxidizable arylamines bind, to a larger extent, to Hb \((28,29)\). The adduct levels found for 24TDA, 24DNT, 26TDA and 26DNT follow this rule. For 24TDA more 4AA2AT is present than 24TDA. 4AA2AT is less oxidizable than 24TDA as demonstrated by the calculation of the stability of the intermediate oxidation product, the nitrenium ion (data not shown). The same argument explains the higher levels of 2AA6AT than 26TDA. For 24DNT, 4A2NT is less oxidizable than 4AA2AT and 24TDA. This corresponds with the highest adduct level found for 4AA2AT. 2A4NT adducts are

Table III. Hb binding of TDA and DNT isomers 24 h after oral administration to female Wistar rats

<table>
<thead>
<tr>
<th>Compound administered to rat</th>
<th>Cleavage producta</th>
<th>Cleaved productsb</th>
<th>Binding pmol/g Hb c</th>
<th>HBIc</th>
<th>HBIc (22) HBIc (19–21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24TDA</td>
<td>23%</td>
<td>187 ± 10</td>
<td>0.024 ± 0.001</td>
<td>-f</td>
<td>0.030</td>
</tr>
<tr>
<td>4AA2AT</td>
<td>77%</td>
<td>613 ± 265</td>
<td>0.080 ± 0.034</td>
<td>-b</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>800 ± 275</td>
<td>0.104 ± 0.036</td>
<td>-f</td>
<td>0.030</td>
</tr>
<tr>
<td>26TDA</td>
<td>19%</td>
<td>1033 ± 93</td>
<td>0.134 ± 0.012</td>
<td>-</td>
<td>0.025</td>
</tr>
<tr>
<td>2AA6AT</td>
<td>81%</td>
<td>4423 ± 555</td>
<td>0.575 ± 0.072</td>
<td>0.15</td>
<td>-b</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>5456 ± 648</td>
<td>0.709 ± 0.084</td>
<td>0.15</td>
<td>0.025</td>
</tr>
<tr>
<td>24DNT</td>
<td>20.5%</td>
<td>4330 ± 615</td>
<td>0.563 ± 0.080</td>
<td>-</td>
<td>0.016</td>
</tr>
<tr>
<td>4A2NT</td>
<td>77.1%</td>
<td>16325 ± 1551</td>
<td>2.122 ± 0.202</td>
<td>0.7</td>
<td>-b</td>
</tr>
<tr>
<td>4AA2AT</td>
<td>2.4%</td>
<td>512 ± 102</td>
<td>0.067 ± 0.013</td>
<td>-f</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>21167 ± 2268</td>
<td>2.752 ± 0.295</td>
<td>0.7</td>
<td>0.016</td>
</tr>
<tr>
<td>26DNT</td>
<td>30.8%</td>
<td>1234 ± 139</td>
<td>0.160 ± 0.018</td>
<td>0.2</td>
<td>0.014</td>
</tr>
<tr>
<td>2A6NT</td>
<td>63.8%</td>
<td>2551 ± 158</td>
<td>0.332 ± 0.020</td>
<td>1.0</td>
<td>-b</td>
</tr>
<tr>
<td>2AA6AT</td>
<td>5.4%</td>
<td>173 ± 75</td>
<td>0.022 ± 0.010</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>3958 ± 372</td>
<td>0.515 ± 0.048</td>
<td>1.2</td>
<td>0.014</td>
</tr>
</tbody>
</table>

a The Hb-cleavage products were identified by GC-MS after base hydrolysis of the Hb.
b The amount of each cleaved Hb product was calculated as a percent of the total Hb-bound material detected.
c Each value is an average from two separate analyses performed on three separate rats. For each compound three female Wistar rats were dosed orally with 0.5 mmol/kg.
d HBI = (mmol compound/mol Hb)/dose (mmol/kg body wt).
e Experiment with female Wistar rats (0.5 mmol/kg).
f Determination limit 0.02.
g Experiment with male Fisher 344 rats (0.41 mmol/kg).
h Not analyzed.
not present. Nitro groups, which are co-planar with the aromatic ring, like the nitro group in the 4 position of 24DNT, are more easily reduced than nitro groups in the ortho position to a methyl group forced out of the plane. The reducibility of nitro groups can be estimated with the LUMO calculated with semi-empirical programs (AM1, PM3). This explains also the difference between the total adduct levels in rats given 24DNT and 26DNT. 24DNT is better reducible than 26DNT. The oxidizability also explains the higher levels of 26TDA compared with 24TDA. 26TDA is less oxidizable than 24TDA.

As shown in Table III there are major differences between the results of the three different studies: the present study, Wilson et al. (19–21) and Zwirner-Baier et al. (22). The results of Wilson et al. (18–20) were obtained in a different rat strain (male Fischer rats) and using a different work-up. Wilson did not use an internal surrogate standard, but an internal volumetric standard 2,6-dimethylaniline, which was added after the hydrolysis and extraction of Hb. Therefore, any differences in extraction or derivatization of 24TDA and 26TDA, would not be accounted for using an internal volumetric standard. In addition Wilson et al. used hydrolysis conditions (concentrated NaOH, 37°C), which would cleave all monoacetylated amines to the parent diamines. In addition, it appears that Wilson et al. did not look for 4A2NT, 2A4NT and 2A6NT. Therefore, only the analyses of the diamines 26TDA and 24TDA were reported. Zwirner-Baier et al. (22) used the same animals and dose regimen to those reported in the present study. The major differences are the extraction procedure, the internal standards and the analysis procedure [HPLC with electrochemical detection (ECD)]. Unfortunately the internal standard used was not specified in the publication (22). In our work we used the deuterated analogs for each arylamine. Therefore, differences in relative recovery of the Hb adducts relative to the internal standard where accounted for in our study. In addition HPLC-ECD methods have in general a higher determination limit than GC-MS-NCI analyses. Therefore, some adducts in animals given 24TDA, 24DNT and 26DNT were missed in the earlier study (22). In summary, only our method allows the analysis of all possible Hb adducts.

**Comparison of rat hemoglobin with human Hb binding**

The Hb-cleavage products detected in hydrolyzed Hb from workers exposed to DNT were qualitatively comparable with the Hb adducts reported in rats administered 24DNT or 26DNT. The predominant Hb-cleavage product in workers exposed to 24DNT was 4A2NT. Low levels of 24TDA, and in some cases similar amounts of the acetylated product, 4AA2AT, were also detected. As in the case of rats administered 24DNT (Table III), mainly reduction of the nitro group para to the methyl group was observed.

In humans the ratio between 4A2NT and 24TDA was very different compared with rats. In humans the ratio for 4A2NT and 24TDA was 24:1 and in rats it was 4:1. Quantitatively, 4A2NT was not as prevalent in rats as in humans. This difference indicates that the concomitant reduction of both nitro groups in man was less prevalent than in rat.

The spectrum of Hb adducts formed in rats and the workers exposed to 26DNT were similar although the acetylated adduct, 2AA6AT was not found in the workers. The quantitative differences between levels of each 26DNT Hb-cleavage product were similar to that described for the 24DNT isomer. In humans the ratio for 2A6NT and 26TDA was 14:1; however, in rats it was 2:1:1. Therefore, also in the case of 26DNT, the concomitant reduction of both nitro groups was more prevalent in rats than in the workers.

To date, only one other group has published data on the fate of DNT in the blood tissues of humans. Neumann et al. (30) quantified DNT-derived Hb adducts in residents living in an area containing soil contaminated with explosives waste and in a control population. 2A4NT, 4A2NT and 2A6NT were analyzed and found at similar levels in the exposed residents and in the control population. The reported adduct levels were higher than in the present study of Chinese workers exposed to DNT. It is peculiar that the general environmental exposure in Germany appears to be higher than the occupational exposure in a Chinese factory. Unfortunately, no experimental details were given for the German study (30,31) and as such the results cannot be reproduced and cannot be discussed further.

To date, Hb adduct determinations had not been described for humans exposed to mono-nitrotoluenes (2NT, 3NT or 4NT) although Hb adducts in rats have been described. The Hb-cleavage products identified in rats administered 0.5 mmol/kg 2NT, 3NT or 4NT were 2MA, 3MA and 4MA (32). The same Hb-cleavage products were identified in hydrolyzed Hb from the Chinese workers.

**Relationship between internal dose of NT and DNT and health effects in Chinese workers**

In previous studies where the health status of workers exposed to DNT was assessed, the most common complaints recorded were due mainly to the ability of DNT to induce Methb, the secondary effects of which were non-specific health effects such as headache, dizziness, nausea and drowsiness (32,33).

A full medical examination was performed on each of the Chinese workers by doctors from the Institute of Occupational Medicine, at the Chinese Academy of Preventative Medicine. Each worker was examined for non-specific adverse health effects linked to exposure to DNT, the results of which have been presented in Table IV. Of particular interest was the investigation of dose–response relationships in the Chinese workers exposed to NT and DNT. We were interested in defining whether biomarkers of recent exposure or chronic exposure correlated with the risk of suffering from one or the other.

**Table IV.** Comparison between the prevalence of adverse health effects induced by exposure to DNT in exposed and factory controls

<table>
<thead>
<tr>
<th>Health effect</th>
<th>Exposed (n = 98)</th>
<th>Control (n = 72)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inertia (feeling of unwillingness to do anything)</td>
<td>31.5%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.4%&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Somnolence (drowsy/sleepy)</td>
<td>16.3%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.8%&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Insomnia</td>
<td>21.4%&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.3%&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Headache</td>
<td>8.2%&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.8%&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dizziness</td>
<td>9.2%&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.4%&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nausea</td>
<td>23.5%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0%&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The number of exposed workers where a symptom was recorded has been calculated as a percent of the total number of exposed workers analyzed. The number of factory control workers where a symptom was recorded has been calculated as a percent of the total number of factory control workers analyzed. The statistical difference of the prevalence between exposed and control workers was tested with the χ² test (likelihood ratio): <sup>a</sup><sub>P < 0.001</sub>, <sup>b</sup><sub>P < 0.01</sub>, <sup>c</sup><sub>P < 0.05</sub>, <sup>d</sup><sub>P > 0.05</sub>.
Hemoglobin adducts in workers exposed to nitrotoluenes

more of the health conditions, symptomatic of toxic exposure to nitroarenes. Logistic regression analysis was used to predict the presence or absence of a particular condition in the Chinese workers. The logistic regression was performed using the log-transformed data of Hb-cleavage products. The obtained odds ratios (OR) are listed in Table V. The OR indicates the odds of suffering for the various health effects of subjects with one log-unit more of each adduct relative to the odds of subjects with one log-unit less. The results presented in Table V show that there is a significant dose–response relationship between the log-transformed values of the aminonitrotoluene Hb-cleavage products and the risk of suffering from inertia, somnolence, dizziness or nausea. The odds of suffering from inertia were 3.2 times higher when the level of 4A2NT Hb adducts increased by one log-unit than the control group (P < 0.001). Similar ORs were observed for somnolence (3.1), nausea (2.4) and dizziness (5.5). The OR of suffering from inertia, somnolence, dizziness or nausea increased by a factor of 3.2, 3.3, 7.8 and 2.7, respectively, based upon the 2A6NT- Hb adduct levels. These results were tested for confounding factors like age, smoker status and gender using stepwise forward logistic regression analysis. In the case of nausea, age is a borderline confounder. The age-adjusted OR increases only by 4, 3.5 and 8.7% for the adducts of 4A2NT, 2A6NT and 24TDA, respectively. Therefore, the crude ORs have been listed in Table V. These results inferred that quantification of DNT–Hb adducts provided an effective biomarker of toxicity and could be used to estimate the risk associated with a particular exposure to DNT.

What is the cancer risk for these exposed workers? In the absence of large epidemiological studies it was necessary to characterize human health risk from animal experiments (34,35). For a risk assessment the daily dose of the Chinese workers has to be estimated. With the knowledge from the animal data it is possible to estimate the daily-dose from the measured Hb adduct levels. However, it is necessary to make the following assumptions: (i) the steady-state level of the Hb adducts is estimated with the following formula (13,36): steady-state adduct level = daily adduct level \times 0.5 \times \text{lifetime of the erythrocytes. Thus, to calculate the single dose the adduct level has to be divided by 60. (ii) Modified Hb has the same lifespan as unmodified Hb and the adducts are stable to repair mechanisms. (iii) The pharmacokinetics of the xenobiotic compound is comparable in rats and humans.

The absorbed dose per day was calculated from the Hb adduct levels using the equation for the steady-state level of Hb adducts. In the rat experiments with 24DNT, 26DNT and 2NT (37), 0.0398, 0.0075 and 0.010435% of the dose were bound to Hb. Assuming that Hb binding in rats and man was comparable, the daily exposure dose of 24DNT, 26DNT and 2NT in workers was estimated. The 95th percentile adduct level of 24DNT, 26DNT and 2NT corresponded to a daily dose of 20.53, 11.02 and 3.46 μg/kg/day, respectively. An excess lifetime cancer risk (ELCR) for exposure to these chemicals was estimated using the formula published by the US regulatory agencies agency (38,39; http://risk.lsd.ornl.gov): (ELCR) = (cancer slope factor) \times (human dose). Workers are not exposed 7 days/week and 52 weeks/year to the workplace contaminant. As we deduced the external dose from the internal dose, days without exposure were included. Therefore, the dose was not corrected for the days without exposure. As workers are not exposed for a lifetime of 70 years to the occupational pollutants, but for 40 years of work life, the dose was corrected with the factor 40/70. The cancer slope factors for 24DNT (40; www.epa.gov/iris), 26DNT (40) and 2NT (41) are 0.68, 0.68 and 0.23 (mg/kg/day)\(^{-1}\), respectively. Therefore, the excess cancer risk for lifetime exposure to 24DNT, 26DNT and 2NT are 8 in 1000, 4.3 in 1000 and 4.6 in 10 000. Adding up the risks yields 1.3 in 100 for a lifetime exposure and the worst-case scenario. Taking the average adduct levels of the exposed workers the risk for 24DNT, 26DNT and 2NT are 2.5 in 1000, 1.3 in 1000 and 2.5 in 100 000, respectively. A risk of 1 in 10\(^6\) is perceived as the virtual safe dose. In any case the risk resulting from 24DNT and 26DNT exposure is far above these levels. Therefore, the exposures in this group of workers should be drastically reduced. This is in accordance with the German Research Commission (42) who classified DNT mixtures as a Category 2 carcinogen. No MAK value (maximum concentrations at the workplace) was listed as a safe concentration range cannot be given. This is in contrast to the permissible exposure limit (PEL) set by the US Occupational Safety and Health Administration (43). For 24DNT and 26DNT the PEL was set at 1.5 and 1.5 mg/m\(^3\) as an 8-h time-weighted average, respectively. Assuming that a 70 kg worker inhales 9.6 m\(^3\) air/day, and assuming 100% absorption of the dose, the daily permissible exposure dose would be 206 μg/kg/day for

<table>
<thead>
<tr>
<th>Health effect</th>
<th>Hb adduct cleavage product</th>
<th>4A2NT OR(^a) (CI)(^b)</th>
<th>2A6NT OR (CI)</th>
<th>24TDA OR (CI)</th>
<th>26TDA OR (CI)</th>
<th>2MA OR (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inertia (feeling of unwillingness to do anything)</td>
<td>3.2(^c) (1.8–5.8)</td>
<td>3.2(^c) (1.8–5.6)</td>
<td>4.5(^d) (2.1–9.9)</td>
<td>4.8(^e) (1.7–13.1)</td>
<td>14.8(^e) (2.8–77)</td>
<td></td>
</tr>
<tr>
<td>Somnolent (drowsy/sleepy)</td>
<td>3.1(^d) (1.4–6.9)</td>
<td>3.3(^d) (1.4–7.4)</td>
<td>4.7(^d) (1.6–13.5)</td>
<td>3.2(^e) (0.93–11.1)</td>
<td>1.7(^e) (0.43–16.5)</td>
<td></td>
</tr>
<tr>
<td>Insomnia</td>
<td>1.3(^d) (0.91–1.8)</td>
<td>1.3(^d) (0.85–2.0)</td>
<td>1.5(^e) (0.84–2.8)</td>
<td>1.4(^e) (0.56–3.4)</td>
<td>5.2(^e) (1.1–25.0)</td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>1.4(^e) (0.78–2.6)</td>
<td>1.2(^e) (0.62–2.2)</td>
<td>1.6(^e) (0.62–4.2)</td>
<td>1.2(^e) (0.32–4.8)</td>
<td>1.6(^e) (0.16–16.1)</td>
<td></td>
</tr>
<tr>
<td>Dizziness</td>
<td>5.5(^e) (1.3–24.2)</td>
<td>7.8(^e) (1.5–40.4)</td>
<td>9.4(^e) (1.7–52.2)</td>
<td>2.4(^e) (0.50–10.9)</td>
<td>1.3(^e) (0.12–15.6)</td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>2.4(^e) (1.3–4.3)</td>
<td>2.7(^e) (1.4–5.2)</td>
<td>4.6(^e) (1.8–11.9)</td>
<td>4.4(^e) (1.4–14.2)</td>
<td>3.3(^e) (0.64–17.1)</td>
<td></td>
</tr>
</tbody>
</table>

Logistic regression analysis of the log-transformed data was performed to ascertain the increased probability of suffering from any one of these symptoms.

\(^a\)OR, odds ratio.

\(^b\)Confidence interval 95%.

\(^c\)The OR was significant (P < 0.001).

\(^d\)The OR was significant (P < 0.01).

\(^e\)The OR was significant (P < 0.05).

\(^f\)The OR was not significant (P > 0.05).
24DNT, and/or 26DNT. Such exposure levels would yield a risk of 8 in 100. Also for other compounds the PEL values appear to be too high when compared with the cancer risk deduced from the animal experiments (34).

The calculated carcinogenic risk was compared with organ-specific changes. The prevalence of tissue changes (hepatocellular) in the liver of the Chinese workers exposed to nitrotoluenes was evaluated: only 2 out of 105 exposed workers showed a deviation from the norm (>1.0 cm). This ratio was not statistically different with that reported in factory control workers (1 out of 14). For tissue changes in the spleen (splenomegaly) only 1 out of 105 exposed workers compared with 1 out of the 14 factory controls showed a deviation from the norm with regard to spleen size. For these spleen and liver investigations the number of controls was only 14 and not 61 as for the other analyses. However, the analyses of 14 controls showed that the prevalence is not lower than in exposed workers. Based upon the high-calculated carcinogenic risk we would have expected to see a higher number of workers with tissue changes in the liver or spleen, although it was apparent from the epidemiological data that the urinary tract was the principle target tissue in humans. Possibly the studied population was not large enough and/or humans are not so sensitive to these compounds.

Acknowledgements
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References
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