Addendum to the Toxicological Profile for 2,4 and 2,6-Dinitrotoluene

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ADDENDUM for 2,4 and 2,6-Dinitrotoluene

Supplement to the 1998 Toxicological Profile for 2,4 and 2,6-Dinitrotoluene

Background Statement

This addendum to the Toxicological Profile for2,4 and 2,6-Dinitrotoluene supplements the profile that was released in 1998.

Toxicological profiles are developed in response to the Superfund Amendments and Reauthorization Act (SARA) of 1986 which amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). CERCLA mandates that the Administrator of ATSDR prepare toxicological profiles on substances on the CERCLA Priority List of Hazardous Substances and that the profiles be revised "no less often than once every three years." CERCLA further states that the Administrator will "establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" [Title 42, Chapter 103, Subchapter I, § 9604 (i)(1)(B)].

The purpose of this addendum is to provide to the public and other federal, state, and local agencies a non-peer reviewed supplement of the scientific data that were published in the open peer-reviewed literature since the release of the profile in 1998.

Chapter numbers in this addendum coincide with the Toxicological Profile for 2,4 and 2,6-Dinitrotoluene (1998). This document should be used in conjunction with the profile. It does not replace it.

2. HEALTH EFFECTS

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

2.2.1 Inhalation Exposure

2.2.1.8 Cancer Effects

Brüning et al. (1999) studied cases of underground copper-miners exposed to DNT in the area of Mansfeld (Sachsen-Anhalt), part of the former German Democratic Republic. Of 500 miners identified as being exposed, 14 cases of renal cell cancer, 5 cases of bladder carcinoma, and 1 case of renal pelvic carcinoma were identified Their exposure histories were compared with 183 DNT exposed persons without evidence of cancer. Exposures were categorized as low, medium, high, and very high. Results were drawn based on a questionnaire and genotyping by polymerase chain reaction (confounding variables were taken into account). Ninety percent of the cases stated they inhaled DNT from explosions, and 60% said they inhaled DNT daily. No dose-dependent distribution of the 14 renal cell cancer cases over the four exposure categories was found compared with the 183 referent group, but the cases of bladder and renal pelvic carcinoma (urothelial tumor cases) occurred mainly in the high exposure category. The genotyping indicated that the persons with urothelial cancer were all “slow acetylators.” The diagnosis, DNT-exposure category and duration, smoking habits, age at diagnosis, and latency of 20 cancer cases are given in Table 2-1.

Table 2-1 - Subject Characteristics in Miners Exposed to Dinitrotoluene[a]

Cases	Diagnosis[b]	Smoking Habit	DNT-Exposure Duration	DNT-Exposure Category (L,M,H,VH)[c]	Latency (years)	Age at Diagnosis (years)
1	Clear-cell RCC	Non-smoker	1965-1990	M	29	53
2	Clear-cell RCC	Non-smoker	1953-1956	L	42	66
3	Clear-cell RCC	10/day for 33 years	1961-1987	H	33	53
4	Chomophilic RCC	10/day for 17 years	1954-1976	M	40	61
5	Clear-cell RCC	20/day for 30 years	1958-1988	M	35	50
6	Clear-cell RCC	Non-smoker	1963-1988	M	24	52
7	Clear-cell RCC	5/day for 12 years	1952-1986	VH	42	62
8	Clear-cell RCC	20/day for 10 years	1956-1990	VH	37	52
9	Clear-cell RCC	10/day for 26 years	1948-1984	M	36	51
10	Clear-cell RCC	10/day for 30 years	1953-1987	H	41	67
11	Chromophilic RCC	10/day 50 years	1951-1986	H	44	69
12	Clear-cell RCC	10/day for 30 years	1974-1989	L	21	54
13	Clear-cell RCC	Non-smoker	1952-1970	M	44	71
14	Clear-cell RCC	Non-smoker	1950-1972	H	46	62
15	TCC of the renal pelvis	10/day for 27 years	1959-1986	H	31	51
16	TCC of the bladder	10/day for 34 years	1952-1981	VH	38	61
17	TCC of the bladder	50/day for 40 years	1946-1990	H	43	60
18	TCC of the bladder	20/day for 38 years	1952-1975	M	42	57
19	TCC of the bladder	15/day for 45 years	1952-1990	H	43	60
20	TCC of the bladder	30/day for 31 years	1963-1980	H	34	61

[a]Source: Brüning et al. (1999)

[b]RCC, renal cell cancer; TCC, transitional-cell carcinoma

[c]L, low; M, middle; H, high; VH, very high

2.2.2 Oral Exposure

2.2.2.2 Systemic Effects

Hepatic. In a case study by Brüning et al. (1999), 25% of 183 DNT exposed (inhalation and dermal) miners indicated signs of liver disorder (increased values of serum enzymes, γ-glutamyl transpeptidase, glutamic pyruvic transaminase, and/or glutamicoxaloacetic transaminase).

2.2.2.7 Genotoxic Effects

George et al. (1998) studied the modulated genotoxicity of 2,6-DNT by alachlor treatment in ninety five-week-old male 344 Fischer rats. Control rats received 5 weeks of peanut oil by gavage while the experiment group received a daily oral dose (50 mg/kg bw) of alachlor in peanut oil. At weeks 1, 3, and 5 of alachlor treatment, rats were gavaged with a single 75 mg/kg DNT dose (in DMSO) or a DMSO treatment alone. Urine samples were taken 24 hours after administration of DNT or DMSO. After incubation with sulfatase and β-gluconidase, urine samples were concentrated by C-18 solid phase extraction, dried under N_2, and prepared for bioassay in *Salmonella typhimurium* TA98. Peanut oil- and alachlor-treated urine samples were not mutagenic, but DNT-treated rats excreted mutagenic urine metabolites. Metabolic activation with S9 in the bioassay decreased the urine mutagenicity. DNT-associated DNA adduct formation significantly increased following 3 weeks of 50 mg/kg alachlor treatment. Compared to controls, alachlor treatment produced a significant difference in the excretion of mutagenic urine metabolites (339 ± 28 vs. 690 ± 130 revertants/ml;$P<0.05$).

2.2.3 Dermal Exposure

2.2.3.2 Systemic Effects

Hepatic Effects. In a case study by Brüning et al. (1999), 25% of 183 DNT exposed (inhalation and dermal) miners indicated signs of liver disorder (increased values of serum enzymes, γ-glutamyl transpeptidase, glutamic pyruvic transaminase, and/or glutamicoxaloacetic transaminase).

2.2.3.8 Cancer Effects

Harth et al. (2005) studied three cases of urothelial cancer in former employees of a chemical factory which manufactured DNT among other chemicals. The highest exposure to DNT occurred during the maintenance and cleaning of DNT machinery, involving extensive skin contact:

- Case 1 transported the explosives in barriers, mixed the explosives, operated the forklift and drove the explosives with exposure to DNT (and other chemicals) for a total of 19 years. He developed urothelium carcinoma of the bladder that was diagnosed 9 years later.
- Case 2 transported raw materials and explosives, supervised acid preparation, transportation, and sampling with exposure to DNT (and other chemicals) for a period of 18 years. Case 2 developed carcinoma of the urinary bladder that was diagnosed 6 years later.
- Case 3 was exposed to DNT from 1963–1990 at the factory where he worked in the boiler house, mixed explosives manually, supervised mixing, formulated DNT, prepared machines for mixing, supervised machines, and repaired machines. Case 3 developed solid metastatic urothelial cancer of the urinary bladder, and he died in 1991.

As reported in Section 2.2.1.8, Brüning et al. (1999) studied cases of underground copper-miners exposed to DNT in the area of Mansfeld (Sachsen-Anhalt), part of the former German Democratic Republic, in which 14 cases of renal cell cancer, 5 cases of bladder carcinoma, and 1 case of renal pelvic carcinoma were identified. Many of the miners reported direct skin contact with DNT.

2.3 TOXICOKINETICS

2.3.1 Absorption

2.3.1.3 Dermal Exposure

Reifenrath et al. (2002) employed an *in vitro* skin penetration-evaporation model using pig skin to predict human skin absorption of organic nitro compounds, including ^{14}C-radiolabeled 2,4- and 2,6-DNT. Two different soils labeled “Yolo” and “Tinker” as well as an acetone solution were spiked with the chemicals and applied to excised pig skin samples. Yolo contained 34% sand, 46% silt, 20% clay, and 1.9% total carbon. Tinker contained 57% sand, 38% silt, 5% clay, and 9.5% total carbon. See Table 2-2 for mean soil and chemical doses. *In vivo* percutaneous penetration was predicted by adding penetrant residues found in the dermis to the amount of penetrant found in receptor fluid. Absorptions were higher for compounds applied in acetone compared to soils and absorptions from low-carbon soil were higher than for high-carbon soil (see Table 2-3). For the soil samples, the majority of the compound was recovered as skin decontamination with much lower amounts measured in the epidermis, dermis, and receptor fluid

(see Table 2-4). The absorptions of 2,4- and 2,6-DNT in low-carbon soil (Yolo) was exceptionally high (15-16%) compared to the other organic nitro compounds tested (5% or less).

Table 2-2 - Soil and Chemical Doses for 2,4- and 2,6-dinitroluene Applied to Pig Skin[a]

Sample	Soil Dose (mg/cm^2)[b]	Chemical dose ($\mu g/cm^2$)
2,4-dinitrotoluene/Tinker	11.0 ± 1.1	6.8 ± 0.7
2,4-dinitrotoluene/Yolo	11.9 ± 0.8	11.8 ± 0.8
2,4-dinitrotoluene/acetone	---	11.5 ± 0.2
2,6-dinitrotoluene/Tinker	17.4 ± 1.6	6.1 ± 0.5
2,6-dinitrotoluene/Yolo	10.7 ± 1.0	9.7 ± 0.9
2,6-dinitrotoluene/acetone	---	9.6 ± 0.1

[a]Source: Reifenrath et al. (2002)
[b]Values represent the mean ±SD of three to six replicates.

Table 2-3 – Percutaneous Adsorption of 2,4- and 2,6-dinitrotoluene in Soils and Acetone[a]

Compounds	Acetone Solution	Yolo soil	Tinker soil
2,4-dinitrotoluene	36 ± 8[b]	15 ± 6	5.4 ± 1.4
2,6-dinitrotoluene	24 ± 6	16 ± 5	3.8 ± 1.6

[a]Source: Reifenrath et al. (2002)
[b]Values represent the mean ±SD of five to six replicates and are expressed as a percentage of applied dose.

Table 2-4 – Disposition of Radioactivity Following Application of 2,4- and 2,6-dinitrotoluene[a]

		Percent applied radioactive dose					
Compound	Vehicle	Evaporation	Skin decontamination[b]	Epidermis	Dermis	Receptor fluid	Total recovery
2,4-dinitrotoluene	Tinker	4.3 ± 1.8[c]	78 ± 16	0.6 ± 0.3	0.18 ± 0.08	5.3 ± 1.3	104 ± 8
2,4-dinitrotoluene	Yolo	2.4 ± 0.4	50 ± 7	1.7 ± 1.0	0.27 ± 0.11	14 ± 6	77 ± 5
2,4-dinitrotoluene	Acetone	35 ± 8	7.1 ± 1.9	5.0 ± 1.8	3.0 ± 2.2	34 ± 6	85 ± 3
2,6-dinitrotoluene	Tinker	5.5 ± 2.0	81 ± 6	0.63 ± 0.15	0.10 ± 0.02	3.7 ± 1.6	99 ± 6
2,6-dinitrotoluene	Yolo	5.5 ± 2.7	46 ± 4	2.5 ± 1.0	0.43 ± 0.19	15 ± 5	85 ± 5
2,6-dinitrotoluene	Acetone	41 ± 8	9 ± 3	5.1 ± 1.4	1.6 ± 0.60	22 ± 5	80 ± 3

[a]Source: Reifenrath et al. (2002)
[b]Skin decontamination procedure consisted of removing soil particles from the skin by vacuuming the surface with a small tube connected to a vacuum pump and then the application of two tape strips.
[c]Values represent the mean ±SD of five to six replicates and are expressed as a percentage of applied dose.

2.3.3 Metabolism

Jones et al. (2005b) studied DNT exposure in relation to excreted metabolites by Chinese TNT manufacturing workers (Liaoning Province, China) in order to develop a method that could be used to biomonitor DNT exposure. Dinitrobenzoic acid (99 % of exposed workers) was the primary metabolite for 2,6-DNT and aminonitrobenzoic acids were the most predominant metabolites for 2,4-DNT exposure.

Jones et al. (2005a) studied DNT exposure in relation to excreted metabolites by Chinese DNT manufacturing workers (Liaoning Province, China) in order to develop a method to measure hemoglobin (Hb) adducts in biological samples. Groups of 99 exposed and 61 non-matched, non-exposed controls DNT workers were given questionnaires inquiring about exposure history, health status, smoking and alcohol consumption, and previous medical records and present symptoms. Based on a study in rats, it was confirmed that >93 % of adduct levels detected in extracts from based hydrolyzed Hb directly resulted from the cleavage of the sulphimic acid amide bond between the cysteine residues on Hb and the arylamine. Hb adducts were determined based on the level of arylamine-cleavage products following mild hydrolysis. Hb adducts were found in a significantly higher amount in exposed workers than in the controls. The count of Hb adducts resulting in the exposed population linked to 2,4-DNT (71.0±68.9 pmol/g [51.9,18.6, and 84.3)] are 11-fold higher than the Hb adducts found in the exposed population linked to 2,6-DNT [6.5±6.5 (4.3,2.1,8.2)]. Aminonitrotoluenes (specifically 4A2NT, present in 99% of those exposed) gave the strongest indication of DNT exposure (r=0.94).

2.3.4 Elimination and Excretion

Jones et al. (2005b) studied DNT exposure in relation to excreted metabolites by Chinese TNT manufacturing workers (Liaoning Province, China) in order to develop a method that could be used to biomonitor DNT exposure. An exposed (n=98, average age 36.2±9.5) and control (n=72, average age 37.4±8.7) group were both given questionnaires inquiring about exposure history, health status, smoking and alcohol consumption, and previous medical records and present symptoms. A total of 126 spot urine samples were collected (115 from exposed workers, 11 from non-matched, non-exposed controls). Air monitoring revealed that the mean TWA 8-hour exposure levels of the exposed workers were 0.043 and 0.014 mg m^{-3} for 2,4-DNT and 2,6-DNT respectively. Optimization of the enzymatic hydrolysis procedure with β-glucuronidase, generation of diazomethane for methylation of benzoic acids, determination of mono- and dinitrotoluene metabolites in urine, quantification of mono- and dinitrotoluenes in metabolites, calibration of the urinary metabolites, determination of the creatinine levels, and supplemental air monitoring for mono- and dinitrotoluenes were all performed in the course of this study. Air measurements did not accurately predict the levels of the urinary metabolites. The 2,6-DNT metabolites were 4-fold lower in the pre- than post-shift. The 2,4-DNT metabolites were 8-fold lower in the pre- compared to post-shift. The metabolites from 2,4-DNT exposure were 2,4-nitrobenzylalcohol, 2-amino-4-nitrobenzoic acid, 4-amino-2-nitrobenzoic acid and 2,4-dinitrobenzoic acid and were present in 89, 88, 91, and 78% of the exposed workers. The metabolites from 2,6-DNT were

2,6-dinitrobenzylalcohol and 2,6-dinotrobenzoic acid, found in 99 and 86% of the workers. The levels of the 2,4-DNT metabolites were 1.4-fold higher than the levels of metabolites resulting from 2,6-DNT exposure A strong correlation existed between the dinitrobenzyl alcohols and the totals level of DNT metabolites, indicating that these alcohols predict the absorbed dose of DNT excreted in worker's urine.

2.4 MECHANISMS OF ACTION

2.4.2 Mechanisms of Toxicity

Sayama et al. (1998) tested the mutagenicities of 2,4- and 2,6-dinitrotoluene plus the corresponding reduced metabolites formed by the incubation of 2,4- and 2,6-dinitrotoluene with *Salmonella typhimurium* TA98 using the Ames test with *S. typhimurium* YG strains possessing high levels of nitroreductase (NR) and/or *O*-acetyltransferase (OAT) activities. Strains YG1041 and YG1042, which possess high levels of NR and OAT activities, yielded the highest mutagenic activities for all compounds tested (~2100-6500 His^+ revertants per µmole) when compared to the other strains (<1000 His^+ revertants per µmole), YG1021, YG1024, YG1026, and YG1029, which only possess NR or OAT activities but not both. This indicates that 2,4- and 2,6-dinitrotoluene need high levels of NR and OAT activities to exert their mutagenicities.

2.5 RELEVANCE TO PUBLIC HEALTH

Genotoxic effects **.**Ozturk and Durusoy (1999) tested two standard genotoxicity assay systems, Quillardet and Hofnung's SOS chromotest using *Escherichia coli* strain PQ37 both in the presence and absence of microsomal (S9) supplements and the *Salmonella typhimurium umu* tester strains NM2009 and NM3009, which express high levels of *O*-acetyltransferase (O-AT) and O-AT plus nitroreductase (NR) respectively. 2,4-DNT tested weakly positive in strain NM3009, but negative in NM2009 and the SOS chromotest, though it displayed a dose-response relationship in NM2009. A maximum dose of 10 µL/assay was used with an exposure time of 3.5h. 2,4-DNT and 2,6-DNT showed weak mutagenic potential by showing increased reverted wells only with *S. typhimurium* TA100 with metabolic action (Neuwoehner et al. 2007).

A study by Padda et al. (2003) examined the mutagenicity and stability of 2,4- and 2,6-DNT and their related transformation products. The mutagenicity of the compounds was tested in *Clostridium acetobutylicum* crude cell extracts using *Salmonella typhimurium* TA100. The Ames test found 2,4- and

2,6-dinitrotoluene to be non-mutagenic but the hydoxylamino first-step intermediates, 2-hydroxylamino-4-nitrotoluene, 4-hydroxylamino-2-nitrotoluene, and 2-hydroxylamino-6-nitrotoluene formed during anaerobic transformation were proven to be mutagenic. Concentrations ranged from 1 μ/mL to 150 μ/mL and showed a linear response up to 100 μ/mL. Past 100 μ/mL, revertants decreased due to apparent toxicity of the sample. The major second-step intermediates, 2,4 and 2,6-dihydroxylaminotoluene, were found to be oxygen sensitive and were not suitable for mutagenic testing. The final metabolites of anaerobic degradation, 2,4-diaminotoluene and 2,6-aminotoluene, were determined to be nonmutagenic.

2.7 BIOMARKERS OF EXPOSURE AND EFFECT

2.7.1 Biomarkers Used to Identify or Quantify Exposure to Dinitrotoluene

Sabbioni et al. (2006) investigated workers exposed to high levels of nitrotoluenes including 2,4- and 2,6-DNT. The exposed (n = 104) and control (n = 72) workers were employed in a factory manufacturing dinitrotoluenes and TNT. The external dose (air levels), the internal dose (urine metabolites), the biological effect dose [hemoglobin (Hb) adducts and urine mutagenicity], and biological effects (chromosomal aberrations and health effects) were assessed. Individual susceptibility was determined though genetic polymorphisms of enzymes assumed to function in nitrotoluene metabolism including glutathione *S*-transferases (GSTM1, GSTT1, GSTP1), *N*-acetyltransferases (NAT1, NAT2), and sulfotransferases (SULT1A1, SULT1A2).

The study found no correlation between air levels and urinary metabolites. The Hb-adducts correlated with the urine metabolites but not with air levels (see Table 2-5). In young subjects (<31 years), the frequency of chromosomal aberrations was increased ($P < 0.05$) in the exposed workers in comparison with the control workers. The GSTM1-null genotype was significantly more prevalent in control workers; the investigators speculated that it is possible that adverse effects (such as nausea) deter GSTM1-deficient subjects from working with nitrotoluenes. Statistically significant effects were seen for on chromosomal aberrations in exposed workers.

Table 2-5 – Comparison of mean (±SD) levels of nitrotoluenes in workplace air, their metabolites in urine, and Hb-adducts in the exposed workers[a]

Exposing agent	Air: TWA concentration (µg/m^3)	Urine metabolites	µmol/L urine	Hb-adducts	Pmol/g Hb
2,4-dinitrotoluene	43.1 ± 98.0	4A2NBA	3.35 ± 5.09	4A2NT	68.3 ± 50.4
		2A4NBA	3.13 ± 6.26	24TDA	2.74 ± 2.41
		24DNBAlc	0.71 ± 1.30		
		24DNBA	0.13 ± 0.18		
2,6-dinitrotoluene	13.9 ± 27.6	26DNBA	4.26 ± 7.42	2A6NT	6.20 ± 6.40
		26DNBAlc	1.38 ± 2.22	26TDA	0.31 ± 0.37

[a]Source: Sabbioni et al. (2006)

TWA = 8-hour time weighted average

2.7.2 Biomarkers Used to Characterize Effects Caused by Dinitrotoluene

The study by Sabbioni et al. (2006) discussed above in Section 2.7.2 is also relevant to this section because it demonstrated that urinary metabolites of DNT correlated with chromosomal aberrations.

2.8 INTERACTIONS WITH OTHER CHEMICALS

The study by Sabbioni et al. (2006) discussed above in Section 2.7.2 is also relevant to this section because it indicated that workers with SULT1A1, SULT1A2, NAT1, GSTT1, GSTP1 genotypes may be more susceptible to chromosome aberrations resulting from nitrotoluene exposure.

3. CHEMICAL AND PHYSICAL INFORMATION

No updated data.

4. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

No updated data.

5. POTENTIAL FOR HUMAN EXPOSURE

5.3 ENVIRONMENTAL FATE

5.3.2 Transformation and Degradation

5.3.2.2 Water

2,4- and 2,6-DNT and their intermediates were prepared in a deionized solution and exposed to the air (Padda et al 2003). The results showed that both 2,4- and 2,6-DNT were stable up to 2,000 min. 4-hydroxylamino-2-nitrotoluene was the most stable of the metabolites while 2-hydroxylamino-4-nitrotoluene (a minor intermediate of 2,4-DNT) and 2-hydroxylamino-6-nitrotoluene (the only intermediate of 2,6-DNT) were less stable. Both 2,4 and 2,6-dihydroxylaminotoluene could not be tested adequately due to their lability in the presence of oxygen.

Nipper et al. (2004) assessed the photo-transformation of 2,6-DNT under simulated solar radiation in seawater solutions. There was an 89% loss of 2,6-dinitrotoluene after 24 hours with none remaining after 72 hours (see Table 5-1). Seawater solutions kept under similar conditions, but without UV radiation, lost only 3.2% of 2,6-dinitrotoluene after 92 h, supporting that the loss under simulated solar radiation was due to photo-transformation.

Table 5-1 – Percent Loss of 2,6-DNT with time, under simulated solar radiation[a].

Exposure time (h)	2,6-DNT concentration (µmol/L)	% 2,6-DNT loss
0	870.07	-
1	818.40	5.9
2	778.85	10.5
4	707.88	18.6
6	613.37	29.5
8	545.75	37.3
24	94.00	89.2
48	38.61	95.6
72	Below detection limit	100.0

[a]Source: Nipper et al. (2004)

5.3.2.3 Sediment and Soil

Biotransformation of 2,4- and 2,6-DNT by the psychrophilic indigenous bacteria in deep sea sediments to 2,4- and 2,6-diaminotoluene, respectively, was studied by Yang et al. (2008). The marine sediment samples were taken from Halifax Harbour and incubation was carried out in anaerobic sediment slurries (10% w/v) at 10°C. 2,4-DNT fully disappeared in about 10 days through the stepwise reduction of the –NO2 functional groups to give 2-amino-4-nitrotoluene and 4-amino-2-nitrotoluene, which transformed further to produce 2,4-diaminotoluene in roughly 50 days. 2,6-DNT also fully disappeared in about 10 days but only took about 35 days for the intermediate, 2-amino-6-nitrotoluene, to fully transform to 2,6-diaminotoluene.

Disappearance of 2,4- and 2,6-DNT occurred in two separate processes. A rapid process accounting for approximately 30% of the total amount occurred first, followed by a second slower process. The initial loss was also observed in the abiotic control samples (which lacked the psychrophilic bacteria), suggesting that the initial loss was attributable to sorption of the chemical to sediment. Addition of lactate to the sediments did not significantly change the removal rate of 2,4- and 2,6-DNT but it did enhance the production of the respective diaminotoluene products, suggesting that sulphate-reducing bacteria are most likely responsible for the transformation of 2,4- and 2,6-DNT.

Biotransformation of 2,6-DNT was assessed by Nipper et al. (2004) in a sandy and fine-grained sediment, with 0.25% and 1.1% total organic carbon, respectively, at 10 and 20 °C. Biotransformation rates occurred in the following sequence: fine grain at 20 °C > fine-grain at 10 °C > sand at 20 °C > sand at 10 °C. In the sandy sediment, all 2,6-DNT was broken down by day 28 at 10 °C and by day 7 at 20 °C. The fine-grained sediment completely broke down by days 7 and 3 at temperatures 10 and 20 °C, respectively. GC/MS analyses indentified 2-amino-6-nitrotoluene as the major transformation product. Like 2,6-DNT, concentrations of 2-amino-6-nitrotoluene dropped faster at the higher temperature and fine-grain sediments. 2-Nitrotoluene, *N*,*N*-dimethyl-3-nitroaniline, benzene nitrile, methylamino-2-nitrosophenol and diaminophenol were identified as minor products. Loss of 2,6-DNT was observed in sterilized sediments suggesting abiotic transformation.

Maeda et al. (2007) reported a correlation between mutagenicity and biodegradability of nitroaromatic compounds, including 2,4- and 2,6-DNT. The umu test with bioluminescent bacteria was used to assess mutagenicity and biodegradability using *Pseudomonas* sp. Strain TM15 bacteria isolated from soil.

Mutagenicity increased with biogradability though 2,4- and 2,6-DNT displayed relatively low rates when compared to aromatic compounds harboring three nitro groups such as 2,4,6-trinitrotoluene, 1,3,5-trinitrobenzene, and 2,4,6-trinitroaniline.

6. ANALYTICAL METHODS

6.2 ENVIRONMENTAL SAMPLES

Zhang et al. (2006) investigated the electrochemical sensitivity of mesoporous MCM-41-modified glassy carbon electrodes to 2,4-dinitrotoluene through cathodic voltammetry. The chemical was dissolved in 0.5M NaCl solution before testing. The method detected concentrations of 2,4-DNT at concentrations as low as 3.4 nM. The study did not state whether lower concentrations were tested or if 3.4 nM was the detection limit. Each voltammogram was recorded within 14 seconds.

In efforts to develop a fast and accurate method to detect landmines, Sylvia et al. (2000) demonstrated the capabilities of surface-enhances Raman spectroscopy (SERS) to detect 2,4-DNT vapor at concentration levels of 5 ppb or less. By misting a gold SERS substrate with a 0.01 M NaOH solution, the team was able to improve detection levels by more than 1 order of magnitude compared to earlier publications. Acquisition times were limited to 30 s to emulate field conditions, but allowing the substrate to sit in the vapor for a longer time interval could possibly lead to greater signals.

Smirnova et al. (2004) reported two crystal structures of DntR (a transcriptional regulator) with acetate and thiocyanate occupying the inducer-binding cavity that optimize the sensitivity of a prototype bacterial cell-based biosensor for the detection of DNTs in soil and groundwater. The structures allow for the construction of models of DntR in complex with salicylate and 2,4-DNT that provide a basis for the design of mutant DntR with enhanced specificity for DNTs.

Albert and Walt (2000) prepared novel cross-reactive optical microsensors for high-speed detection of DNT in water vapor. EPA method 8330 was previously the main detection method for DNT in water vapor, but consumed extensive amounts of time and fiscal resources. DNT was detected at a level of 23 ppb in clean dry air.

Content et al. (2000) developed a method to detect DNT, among other substances, by quenching porous silicon photoluminescence that provides a means to distinguish between nitro containing molecules and

other organic species. The detection is achieved by catalytic oxidation of DNT. The detection limit for DNT is 2 ppb (exposure time of 5 minutes in air).

Albert et al. (2001) developed an instrument to detect levels (120 ppb in blind humidified samples) of DNT based on artificial nose technology using distal tips of an optical fiber bundle. The instrument is used to detect DNT in surface soils, land mines, spike soils, water, and ground samples and capable of distinguishing between background and environmental samples.

Campbell et al. (2003) developed a means of analyzing DNT in soil utilizing pressurized fluid extraction and gas and liquid chromatography-mass spectrometry. As compared with the former EPA method, 15 minutes (compared to 18h) is needed to complete an extraction and MS confirmation of analytes is possible. The limit of quantitation for DNT was 0.05 µg/g.

Honeychurch et al. (2003) developed an electrochemical assay based on spring-printed carbon electrodes for 2,6-DNT that could analyze a 100 µl aliquot with a linear calibration plot from 161 ng ml^{-1} (detection limit) to 137 g ml^{-1}. Media tested were water, saliva samples, and dust wipes with recovery rates of 47.5, 73.4 and 102.4% respectively.

7. REGULATIONS AND ADVISORIES

No updated information.

8. REFERENCES

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