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Isolation and Characterization of TNT and Its Metabolites in Groundwater by Gas Chromatograph-Mass Spectrometer-Computer Techniques

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Disposal of the explosive TNT (2,4,6-trinitrotoluene) and its congeners and degradation products from munitions manufacturing plants and disposal sites presents a serious and potentially hazardous environmental problem. A single manufacturing plant can generate as much as 500,000 gallons of waste water per day (WALSH et al. 1973). In addition to TNT, this water contains other nitro compounds such as dinitrotoluenes, and isomers of TNT. Upon exposure to sunlight and adjustment of pH prior to disposal, these compounds undergo chemical transformation, producing highly colored substances, the identity and toxicity of which are not known. These finishing plant waters are disposed by discharging them into holding lagoons, rivers, or streams. Obsolete munitions containing TNT have been disposed at sea (HOFFSOMMER et al. 1972). In shell-loading operations large volumes of hot water are used to wash out residual explosives. This waste water containing TNT is disposed in local streams (WON et al. 1974).

TNT is toxic to fish at concentrations greater than 2 µg per ml (WON et al. 1974), and in humans TNT has been shown to cause liver damage and anemia (SAX, 1957), (McCORMICK et al. 1976). Recently, TNT has been described as being toxic and mutagenic (WON et al. 1976). In view of the toxicity of TNT and problems associated with its disposal, extreme caution is necessary in order to avoid contamination of ground and surface waters. Although methods exist for the determination of TNT and other explosives in water and soil (GOERLITZ et al. 1972), few attempts have been made to characterize metabolic or degradation products of TNT in contaminated groundwater.

During a study of groundwater contamination by percolating wastes derived from explosives at the Hawthorne Naval Ammunition Depot, Nevada, it was observed that shallow ground waters beneath and downgradient from the disposal beds contain TNT (maximum determined concentration, 620 µg/l) and other organic and inorganic nitrogen-bearing compounds apparently

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related to the percolating wastes (A. S. VAN DENBURGH, U.S. Geological Survey, oral communication). This present report describes the isolation and characterization of 2,4,6-trinitrotoluene (TNT), 2,4-dinitrotoluene (2,4-DNT), and two metabolic degradation products, 4-amino-2,6-dinitrotoluene (4-Am-DNT) and 2-amino-4,6-dinitrotoluene (2-Am-DNT), in contaminated groundwater at this site.

METHODS AND MATERIALS

Isolation of TNT and Metabolites from Ground-Water

One liter of the ground-water sample was extracted with benzene (3 X 50 ml). The combined benzene extracts were dried for 2 hr over anhydrous sodium sulfate, then filtered through glass wool into a Kuderna-Danish apparatus and concentrated to 1.0 ml. The extract was then subjected to column chromatography on a deactivated alumina column, and eluted with benzene. The benzene eluate was concentrated to 1 ml with a stream of dry nitrogen. Five microliters of this solution was injected into the electron capture gas chromatograph for preliminary screening purposes.

Equipment

A Tracor Model 550[1] gas chromatograph equipped with a Ni-63 electron-capture detector was used for relative retention time studies. Samples were chromatographed isothermally at 180°C on 6' X 1/4" glass columns packed with either 3% OV-101 on Gas Chrom Q, 100/120 mesh, or 1.5% SP-2250/1.95% SP-2401 on 100/120 Supelcoport. Ar/CH_4:95/5, V/V was used as carrier gas at a flow rate of 60 ml/min.

GC-MS analyses were performed on a Finnigan Model 4010 GC-MS-Data System[1]. The GC column was packed with 3% OV-17 on Gas Chrom Q, 100/120 mesh. Helium at a flow rate of 30 ml/min was used as carrier gas. A 3-µl aliquot of the benzene extract was injected into the gas chromatograph (oven temperature 190°C); and after a one-minute delay, the oven was programed at 6°C/min (to 240°C). Data acquisition was commenced simultaneously with temperature programming. The mass spectrometer was operated using an ionizing voltage of 70 ev and an ionizing current of 250 µA.

[1] The use of the brand names in this report is for identification purposes only and does not imply endorsement by the U.S. Geological Survey.

RESULTS AND DISCUSSION

Figure 1 shows an electron capture gas chromatogram of the contaminated ground-water sample extract.

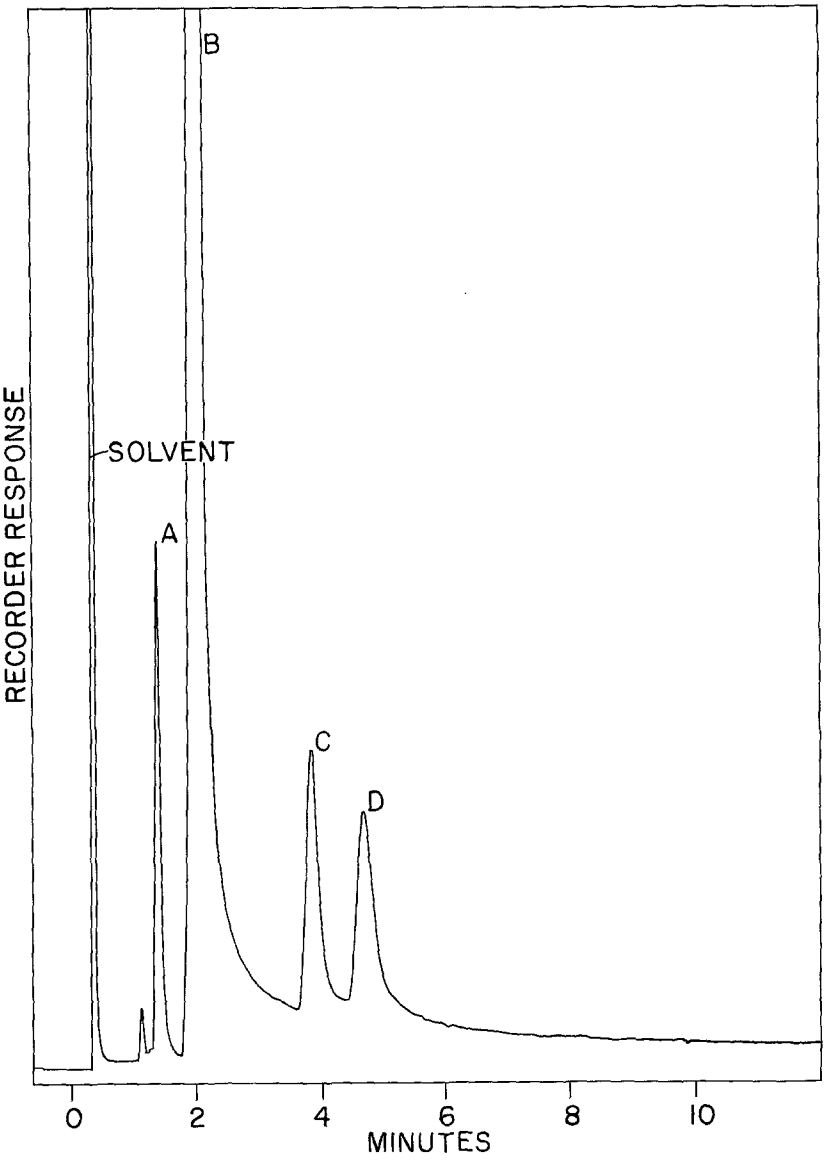

Fig. 1. Electron capture gas chromatogram of contaminated groundwater sample extract. A, 2,4-DNT; B, TNT; C, 4-Am-DNT; D, 2-Am-DNT. Column 3% OV-101.

The chromatogram in Fig. 1 shows essentially four major peaks. GC-MS analysis revealed that the four peaks correspond to A) 2,4-dinitrotoluene; B) 2,4-6-trinitrotoluene; C) 4-amino-2,6-dinitrotoluene; D) 2-amino-4,6-dinitrotoluene. The identity of these compounds was confirmed by comparison of their mass spectra and relative retention times with those of authentic standards analyzed under identical conditions.

Table I shows the relative retention times (RRT) of authentic standards on two columns. The relative retention times of the compounds found in the contaminated ground-water sample is shown in Table II.

TABLE I. Relative retention times of standards obtained with an isothermal column (180°C)

	RRT vs Aldrin		RRT vs TNT	
Compound	OV-101	SP-2250/ SP-2401	OV-101	SP-2250/ SP-2401
2,4-dinitrotoluene	0.27	0.50	0.70	0.64
2,4,6-trinitrotoluene (TNT)	0.38	0.77	1.00	1.00
4-amino-2,6-dinitrotoluene (4-Am-DNT)	0.72	1.95	1.88	2.72
2-amino-4,6-dinitrotoluene (2-Am-DNT)	0.85	2.60	2.24	3.40

Figure 2 shows a total ion chromatogram of the contaminated groundwater sample extract.

The mass spectra of compound (A) 2,4-dinitrotoluene and (B) 2,4-6-trinitrotoluene were in good agreement with those published in the literature (KARSEK, 1974). To the best of our knowledge, the mass spectra of compound (C) 4-amino-2,6-dinitrotoluene and compound (D) 2-amino-4,6-dinitrotoluene have not been published. A computerized library search of the Mass Spectral Search System (MSSS) data bank (HELLER et al. 1975) for compounds (C) and (D) did not produce any positive matches. The identity of these compounds was confirmed by comparison of their mass spectra with those of authentic standards.

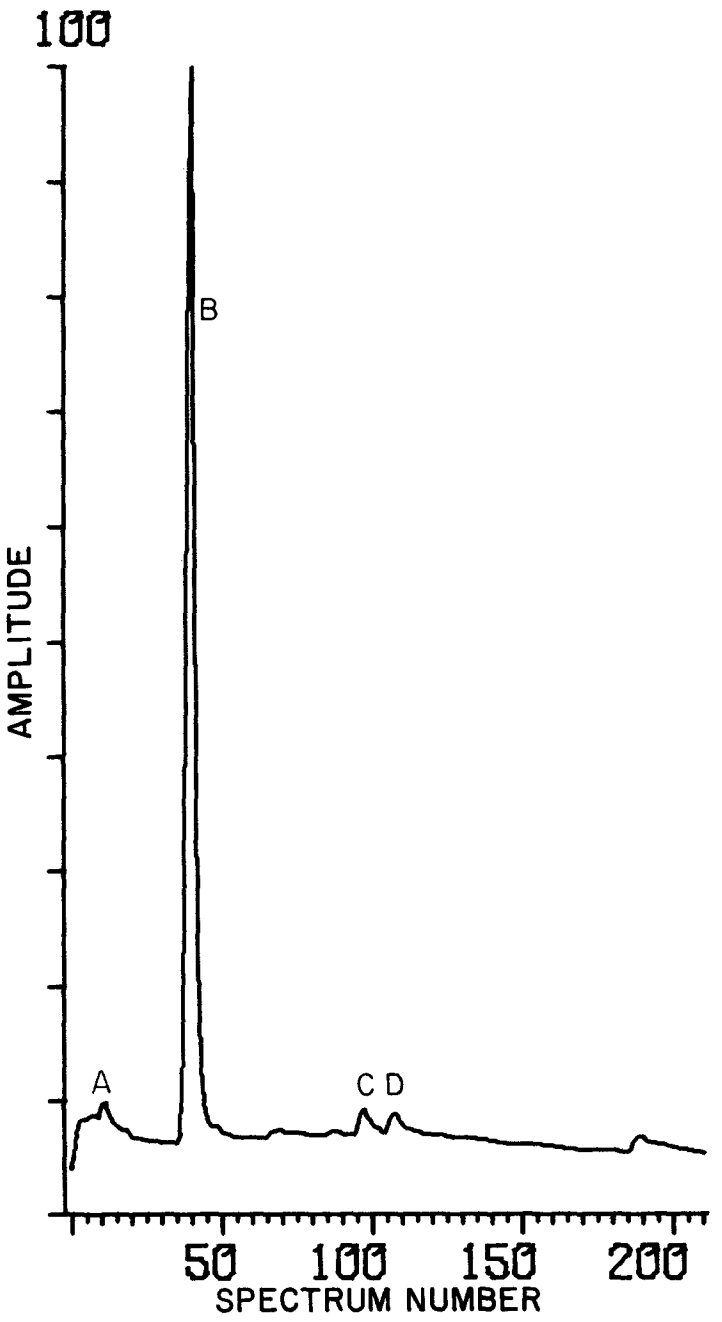

Fig. 2. Total ion chromatogram of the contaminated groundwater sample extract.

TABLE 2. Relative retention times of trinitrotoluene (TNT) and its metabolites (isolated from a ground-water sample) obtained with an isothermal column (180°C)

Compound	RRT vs Aldrin		RRT vs TNT	
	OV-101	SP-2250/ SP-2401	OV-101	SP-2250/ SP-2401
2,4-dinitrotoluene	0.27	0.50	0.70	0.65
2,4,6-trinitrotoluene (TNT)	0.39	0.77	1.00	1.00
4-amino-2,6-dinitrotoluene (4-AM-DNT)	0.71	1.98	1.88	2.59
2-amino-4,6-dinitrotoluene (2-Am-DNT)	0.86	2.60	2.27	3.50

Figure 3 shows the mass spectra of an authentic standard of 4-amino-2, 6-dinitrotoluene and that of compound (C) in contaminated groundwater.

Figure 4 shows the mass spectra of an authentic standard of 2-amino-4,6-dinitrotoluene and that of compound (D) in contaminated groundwater sample.

The presence of 2,4-dinitrotoluene in the sample is not surprising since this compound has been identified as an impurity in crude TNT (TEH-LIANG CHANG, 1971). 4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene are probably formed by bacterial degradation of TNT. The metabolic degradation of TNT in fungi, bacteria, and mammalian systems involves a stepwise reduction of the nitro groups, through the intermediate nitroso and hydroxylamino, to the amino groups (TAKAHASHI *et al.* 1963). It is interesting to note that compounds (C) and (D) have also been found in the urine of workers from munitions factories (HASSMAN, P., 1971); LEMBERG *et al.* 1944). Our results are consistent with the work of WON (WON *et al.* 1974) who reported that pseudomonad-like organisms, growing in a medium supplemented with glucose and yeast extract, converted TNT to these reduction products. The TNT molecule is apparently capable of being reduced in both aerobic and anaerobic systems (McCORMICK *et al.* 1976).

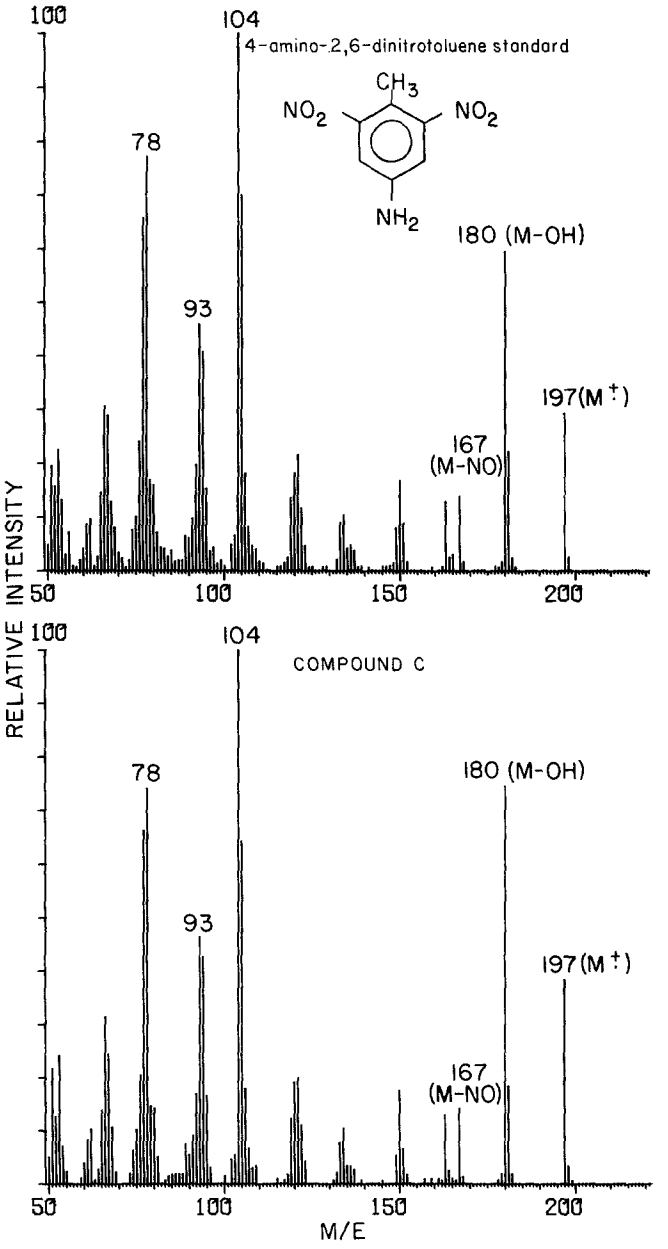

Fig. 3. Mass spectra of 4-amino-2,6-dinitrotoluene standard and that of compound C in contaminated groundwater sample.

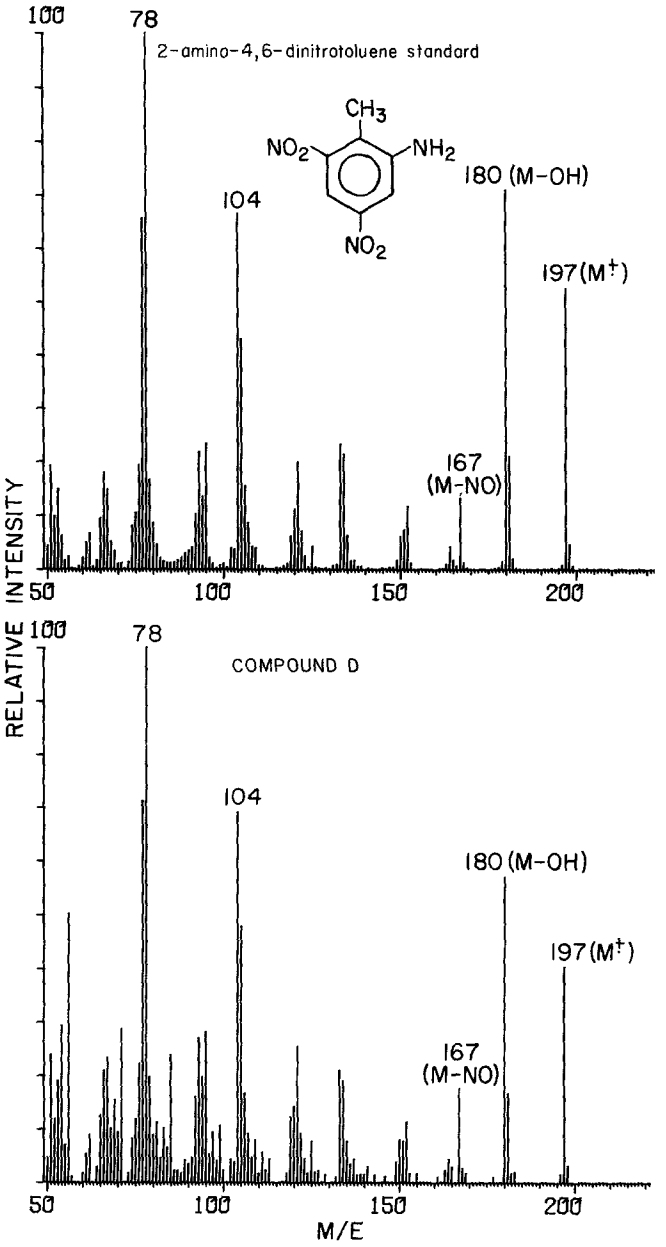

Fig. 4. Mass spectra of 2-amino-4,6-dinitrotoluene standard and that of compound D in contaminated groundwater sample.

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