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Biodegradation of Hexahydro-1,3,5-Trinitro-1,3,5-Triazine

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Biodegradation of the explosive hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) occurs under anaerobic conditions, yielding a number of products, including: hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine, hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine, hexahydro-1,3,5-trinitroso-1,3,5-triazine, hydrazine, 1,1-dimethylhydrazine, 1,2-dimethylhydrazine, formaldehyde, and methanol. A scheme for the biodegradation of RDX is proposed which proceeds via successive reduction of the nitro groups to a point where destabilization and fragmentation of the ring occurs. The noncyclic degradation products arise via subsequent reduction and rearrangement reactions of the fragments. The scheme suggests the presence of several additional compounds, not yet identified. Several of the products are mutagenic or carcinogenic or both. Anaerobic treatment of RDX wastewaters, which also contain high nitrate levels, would permit the denitrification to occur, with concurrent degradation of RDX ultimately to a mixture of hydrazines and methanol. The feasibility of using an aerobic mode in the further degradation of these products is discussed.

Among pollutants unique to the military are those arising from the manufacture, handling, and demilitarization of munitions. Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is an explosive widely used for military purpose. A homolog, octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), is a by-product of the synthesis of RDX and is also used in RDX formulations. During the manufacture of RDX, up to 12 μg/ml may be discharged to the environment in process wastewaters (14). The question of the toxicity of RDX to aquatic life has been addressed (6, 23), and studies conducted by the Office of The Surgeon General have recommended a 24-h average maximum allowable concentration of 0.30 μg of RDX per ml of wastewater to protect aquatic life (23). It has not been clearly established whether or not RDX is toxic in mammalian toxicity studies (11, 16, 17). These reports suggest that RDX per se may not present a serious toxicity problem but that metabolic products derived from RDX may be toxic.

A number of studies on the products formed from the chemical decomposition of RDX have been reported. Hoffsommer et al. (13) reported that the alkaline hydrolysis of RDX yielded nitrate, nitrogen, ammonia, nitrous oxide, formic acid, formaldehyde, and traces of H_2. The first step in this reaction was a proton abstraction by the base with simultaneous elimination of a nitrite group from the adjacent ring nitrogen (4). Exposure of aqueous solutions of RDX to ultraviolet (UV) light resulted in the formation of nitrate, nitrite, ammonia, formaldehyde, nitrous oxide, formamide, and *N*-nitroso-methylenediamine (7). The combination of UV light with ozone produced CO_2, cyanic acid, nitrate, ammonia, and formic acid (8).

The decomposition of RDX in a biological system has been reported. Osmon and Klausmeier (15) found that some RDX disappeared during soil enrichment studies, but evidence for RDX degradation by microorganisms was not obtained. The complete disappearance of RDX by mixed cultures of purple photosynthetic bacteria was reported by Soli (22), who advanced the hypothesis that the strongly reducing conditions of the culture during photosynthesis were responsible for disruption of the RDX molecule. Hoffsommer et al. (12) found no disappearance of RDX in an aerobic activated-sludge system. Sikka et al. (19) reported the disappearance of RDX, after a 20-day lag period, from river water samples supplemented with river sediment. The observation was made that $^{14}CO_2$ was evolved from [^{14}C]RDX depending on the source of the sediment.

The products of the biological degradation of RDX may pose more serious toxicological problems than the RDX itself. Therefore, it is important not only to assess the environmental fate of RDX, but also to determine the nature of the products of biotransformation or biodegradation or both.

MATERIALS AND METHODS

Cultures and media. Biodegradation studies were carried out in nutrient broth (Difco Laboratories, De-

troit, Mich.). For aerobic studies the media were inoculated with activated sludge obtained from the Marlboro Easterly Municipal Sewage Treatment Plant, Marlboro, Mass. The volume of liquid did not exceed 10% of the total volume of the flask; the flasks were incubated at 30°C on a reciprocating shaker (150 strokes/min). For anaerobic studies the vessels were filled to approximately 95% of their capacity, inoculated with anaerobic sewage sludge (obtained from the Nut Island Sewage Treatment Plant, Boston, Mass.), and incubated as stationary cultures at 37°C. The inocula were prepared by diluting the sludge with two volumes of distilled water and filtering through glass wool. A 2% (vol/vol) inoculum was used.

Due to the relative insolubilities of the compounds used as substrates, weighed amounts were added to empty flasks and dissolved in a small amount of acetone. The acetone was evaporated by a stream of N_2, leaving a thin film of material on the inside surface of the flask. The culture medium was deoxygenated by boiling, poured into the flask containing the deposit of material, and stirred vigorously until solution was attained. The medium was allowed to cool to 35 to 40°C before inoculation. Samples were removed from the culture vessels after various periods of incubation and centrifuged, and the supernatant solutions were filtered through 0.22-μm membrane filters. The resulting culture medium filtrates (CMF) were used for all analytical procedures.

Liquid chromatography. Samples of CMF (10 to 20 μl) were injected without further treatment into a Waters model 6000A liquid chromatograph equipped with a μBondapak C-18 column and a model 450 variable wavelength detector (Waters Associates, Inc., Milford, Mass.). The solvent system used to monitor the disappearance of RDX was 20% methanol in water; solvent flow was 2.5 to 3.0 ml/min, UV detector at 230 nm. For determination of formaldehyde, the solvent was 60% methanol in water, UV detector at 340 nm.

Vacuum distillation. Samples of CMF (100 ml) were placed in a 500-ml boiling flask fitted with an adaptor to which an empty flask was attached. The CMF was frozen in liquid N_2, a vacuum (0.01 mmHg, 1.3 Pa) was established, and the system was closed off. The empty flask was placed in liquid N_2, and the sample-containing flask was allowed to warm. After 2 h the system was opened to atmospheric pressure, and the colorless material which had collected in the second flask was assayed for radioactivity and subjected to analysis by gas chromatography-mass spectrometry (GC/MS) for the presence of methanol.

Determination of formaldehyde. CMF (125 ml) was adjusted to pH 2 with H_2SO_4 and distilled. The distillate was collected with the delivery tube submerged below the surface of a small amount of water to retain volatile components. To 100 ml of distillate was added 1 ml of 10% 5,5-dimethyl-1,3-cyclohexanedione (methone) in 95% ethanol. The mixture was heated to boiling for 5 min, cooled, and stored at 4°C for several days to allow crystallization to occur. Crystals were collected, washed with a small amount of ice-cold water, and dried (mp 192°C; literature 189°C [24]). The colorimetric determination of formaldehyde was carried out on 0.1- to 1.0-ml portions of distillate by the chromotropic acid method as described by Grant (9). The absorbance was determined at 570 nm with a Coleman Junior spectrophotometer. A high-performance liquid chromatographic (HPLC) method was developed for determining formaldehyde directly in aqueous systems, based on a method described by Beasley et al. (1) for the determination of formaldehyde in air. The method involved the addition of 1.0 ml of a filtered 0.1% solution of 2,4-dinitrophenylhydrazine in 2 N HCl to 1.0 ml of CMF contained in a small test tube. The contents were mixed and allowed to react for 10 min, and 10 to 20 μl was injected directly into the HPLC. Authentic 2,4-dinitrophenylhydrazone of formaldehyde was synthesized (18) as a reference material (mp 166°C; literature 166°C).

Synthesis of [^{14}C]RDX. The synthesis of [^{14}C]-RDX was based on the method described by Schiessler and Ross (U.S. patent 2,434,230, 1948). The reaction was conducted in the screw-capped vial (60 by 17 mm) in which [^{14}C]paraformaldehyde (1 mCi, 2.3 mg) was received (New England Nuclear Corp., Boston, Mass.). The cap was fitted with a Teflon liner, and 23.4 mg of finely powdered paraformaldehyde was added to the vial to bring the total paraformaldehyde to 0.85 mmol. Finely powdered NH_4NO_3 (68.5 mg, 0.85 mmol) was introduced into the vial, together with a 10-mm Teflon-clad miniature stirring bar. Acetic anhydride (0.3 ml, 3.2 mmol) was added, followed by 50 μl of boron trifluoride etherate. The vial was tightly closed and heated for 7 h at 65 to 70°C with stirring. At the end of the reaction time the vial was opened, and the stirrer was removed and washed with several drops of acetic acid. The product was precipitated by the addition of 0.3 ml of water and brought into solution by boiling the reaction mixture. The product was allowed to crystallize at room temperature for several hours. The supernatant solution was removed by decantation, and the crystals were washed twice with their volume of water. The product was dried under a current of air at 70°C and finally at 0.1 (13.3 Pa) mmHg at 25°C. The yield of product was 28.9 mg (45.9% of theoretical). The infrared spectrum was identical to that of a twice recrystallized authentic sample of RDX obtained from Holston Army Ammunition Plant, Holston, Tenn. Both samples exhibited the same R_f on silica gel thin-layer cochromatography (TLC). A trace of HMX was eliminated by recrystallization from acetone.

Synthesis of TNX. Hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) was prepared by the method of Brockman et al. (3). The crude product was recrystallized twice from 95% ethanol and then from benzene to give 4.8 g of yellow needles (mp 105 to 107°C; literature 105 to 107°C). The recrystallized product gave a single spot when subjected to TLC analysis on Eastman silica gel plates with fluorescent indicator. The developing solvent was benzene-ethanol (95:5), and the material was visualized by fluorescence quenching under UV light. The infrared spectrum was consistent with the structure, exhibiting bands at 1,449 cm^{-1} and 1,495 cm^{-1} (N-NO). The mass spectrum of the product had a peak at m/z 174, corresponding to the molecular ion, and a major fragment ion at m/z 100.

Synthesis of MNX and DNX. Hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) and hexahydro-

1,3-dinitroso-5-nitro-1,3,5-triazine (DNX) were synthesized from TNX by the method described by Simacek (20). The synthesis yielded a mixture of MNX, DNX, and unreacted TNX. MNX and DNX were isolated as a single slow-moving band by preparative TLC and further purified by HPLC. MNX was subjected to analysis by GC/MS and exhibited a molecular ion at *m/z* 206 and a major fragment ion at *m/z* 132. GC/MS analysis of DNX showed a molecular ion at *m/z* 190 and fragment ion at *m/z* 116.

Isolation of hydrazine and its dimethyl derivatives. CMF was made alkaline with NaOH and distilled in a rotary evaporator at 50°C under 15 mmHg (2.0 kPa). The distillate was collected in dilute HCl. The colorless distillate was evaporated to dryness in the same manner to yield residue A. For the isolation of salicylazine, residue A was dissolved in a small volume of water and neutralized with NaOH. The resulting solution was shaken with 25 ml of 4% salicylaldehyde in benzene. The benzene layer was separated and evaporated to dryness at room temperature under a stream of N_2. The residue was stored over silica gel in a vacuum desiccator for several days to remove excess salicylaldehyde. The residue was subjected to TLC analysis with benzene as solvent. A reference sample of salicylazine, prepared by the method of Blout and Gofstein (2), was cochromatographed with the unknown. On visualization with UV light the TLC exhibited two principal spots, the lower of which had the same R_f as salicylazine. The materials was analyzed by GC/MS, and two principal peaks were observed. The larger had the same retention time as salicylazine (molecular weight, 240), exhibiting a molecular ion at *m/z* 240. Its spectrum was identical to that of the reference standard. A small peak with molecular ion at *m/z* 239 was not futher identified. For the isolation of the dimethylhydrazines, residue A was refluxed with 10 ml of methanol and cooled to room temperature. The supernatant was removed to a small vial and evaporated to dryness in a block heated at 70°C. The methanol extraction of residue A was repeated twice more with 3 ml each of methanol. The residue from the final evaporation was treated with 250 μl of acetic anhydride. The vial was sealed with a Teflon-lined cap and heated at 65°C for 10 min. Standards were concurrently prepared with 3 μl each of methylhydrazine, 1,1-dimethylhydrazine, and 1,2-dimethylhydrazine treated in the same manner. An additional standard was prepared containing 3 μl of 1,1-dimethylhydrazine and 10 mg of ammonium chloride in addition to the acetylating mixture. The samples were analyzed with a Bendix model 2500 gas chromatograph with a 6-ft (183 cm) Pyrex column (0.25-in [0.64 cm] diameter) packed with Tenax GC. The carrier gas was N_2 at 40 ml/min; a flame ionization detector was used at 250°C, and the injection port was maintained at the same temperature. With the oven temperature at 230°C, the derivatized extracts from the CMF gave two small peaks. The peak at 4.6 min corresponded to acetyl-1,2-dimethylhydrazine; the other peak at 3.5 min was not identified. No peaks corresponding to acetyl-1,1-dimethylhydrazine (1.6 min) or acetylmethylhydrazine (4.3 min) were observed. However, when the oven temperature was reduced to 190°C a larger peak was observed at 4.1 min, corresponding to acetamide-dimethylhydrazone. This compound is formed from 1,1-dimethylhydrazine under derivatization conditions when an excess of ammonium chloride is present, as was the case in CMF. The identities of acetamide-dimethylhydrazone (*m/z* 101) and acetyl-1,2-dimethylhydrazine (*m/z* 102) were confirmed by GC/MS.

Distribution of radioactivity. CMF was adjusted to pH 3 with HCl and sparged with helium for 2 h. The gas passed sequentially through a series of traps which contained 0.1 M Na_2SO_3, 0.1 N HCl, water, and 0.1 N NaOH, respectively. The sparging gas continued through a copper oxide-packed column heated to 700°C and, finally, through a second 0.1 N NaOH trap. After purging the acidified mixture for 2 h, the mixture was adjusted to pH 11, and the sparging was continued for an additional 2 h. The resulting purged aqueous phase was neutralized and subjected to continuous ether extraction for 24 h. The aqueous phase was adjusted to pH 3 and similarly extracted with ether. Finally, the aqueous phase from the pH 3 ether extraction was adjusted to pH 11 and again extracted with ether for 24 h. Samples (usually 1.0 ml) were placed in scintillation vials to which 10 ml of Aquasol-2 (New England Nuclear) was added. Measurements for radioactivity were carried out in a model 3255 Packard Tri-Carb liquid scintillation spectrometer.

RESULTS

RDX disappearance. RDX at concentrations of 50 or 100 μg/ml disappeared rapidly from nutrient broth cultures inoculated with anaerobic sewage sludge and incubated anaerobically (Fig. 1). RDX disappearance was essentially complete after 4 days. Concentrations of RDX remained unchanged when cultures were inoculated with aerobic activated sewage sludge and incubated aerobically. No RDX disappeared in uninoculated controls.

Intermediate formation. HPLC analysis of anaerobic reaction mixtures revealed the presence of intermediates formed during the disappearance of RDX (Fig. 1). The fact that the reaction proceeded only under anaerobic conditions suggested that these intermediates might be reduced forms of RDX. Chloroform extraction of an 8-day incubation mixture which initially contained 50 mg of RDX per ml yielded yellow crystals having the same melting point, infrared spectrum, and GC/MS as authentic TNX. Extraction of 2- to 3-day-old cultures (appreciable quantities of MNX and DNX present) with ethyl acetate and subsequent 500-fold concentration allowed MNX and DNX to be separated in sufficient quantity by HPLC to establish their identity by GC/MS. As with the synthesized reference compounds, a molecular ion at *m/z* 206 and a major fragment with *m/z* 132 were detected from the compound corresponding to curve B (Fig. 1), and a molecular ion at *m/z* 190 and fragment ion at *m/z* 116 were

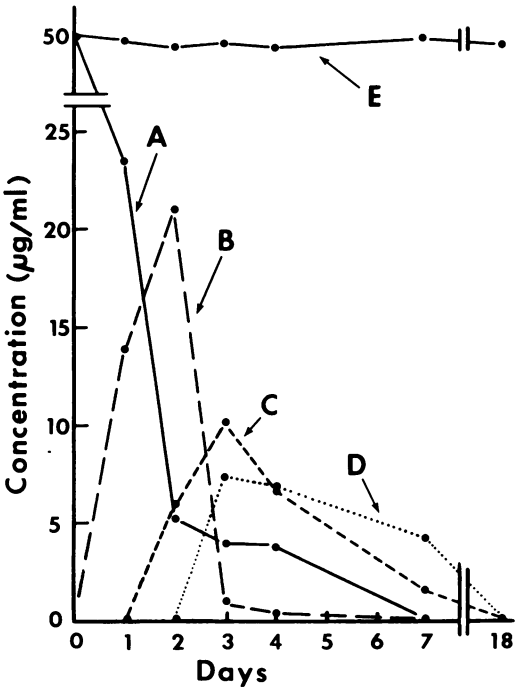

FIG. 1. *Disappearance of RDX and production of intermediates during anaerobic incubation. A, RDX; B, MNX; C, DNX; D, TNX; E, RDX incubated under aerobic conditions.*

detected from the compound corresponding to curve C. The material corresponding to curve D was identified as TNX by TLC, HPLC, and GC/MS, yielding a molecular ion at *m/z* 174 and a major fragment ion at *m/z* 100.

Distribution of radioactivity from [^{14}C]-RDX. A reaction mixture containing 50 mg of RDX per ml, including 2 μCi of [^{14}C]RDX, was incubated anaerobically for 7 days. No ^{14}C-labeled gas was evolved during incubation. The incubated mixture was sparged with helium and extracted with ether as described. The results are shown in Table 1. No more than 1.5% of the total ^{14}C added to the system was found in volatile (spargeable) material. Even after three continuous ether extractions, approximately 42% of the ^{14}C remained in the aqueous phase. Most of this remaining radioactivity disappeared upon evaporation of the sample to dryness at 45 to 50°C under a stream of N_2, regardless of pH. Radioactivity from ^{14}C-labeled RDX was found almost exclusively in the soluble fraction from the earliest measurement (24 h) to the 21st day, with only 2% associated with the pellet or retained by membrane filters.

Formaldehyde. The presence of HCHO in distillates was demonstrated by the formation of the dimethone derivative and by the characteristic and specific color reaction obtained with chromotropic acid. The amount of HCHO produced from RDX increased to a maximum after 1 to 2 days of incubation (Fig. 2), after which the concentration declined to a negligible value.

Methanol. The total ^{14}C found in the distillate could not be reconciled with the specific activity expected if all of the ^{14}C in the distillate was present as HCHO. However, initial attempts to identify and quantitate the other ^{14}C-containing material led to the loss of most of the radioactivity. Since the entire reaction was the result of an anaerobic process, there was the possibility that the polar, volatile, low-molecular-weight, carbon-containing compound might be methanol. Radioactive CMF from reaction mixtures which had been incubated 2 to 3 weeks were subjected to vacuum distillation. Radioactive material collected in a liquid N_2 trap was analyzed by GC/MS. The presence of up to 300 μg of MeOH per ml (9 mM) in the distillate was confirmed. Kinetic data on the formation of MeOH are not yet available.

TABLE 1. *Distribution of radioactivity after anaerobic incubation with [^{14}C]RDX*

Treatment	Radioactivity (%)
None	100.0
Volatiles (from traps)	1.5[a]
Ether extraction, pH 7[b]	23.7
Ether extraction, pH 3[b]	25.4
Ether extraction, pH 11[b]	7.8
Aqueous phase	41.6

[a] Total radioactivity recovered from purging volatiles under acid and alkaline conditions.
[b] Continuous ether extraction for 24 h.

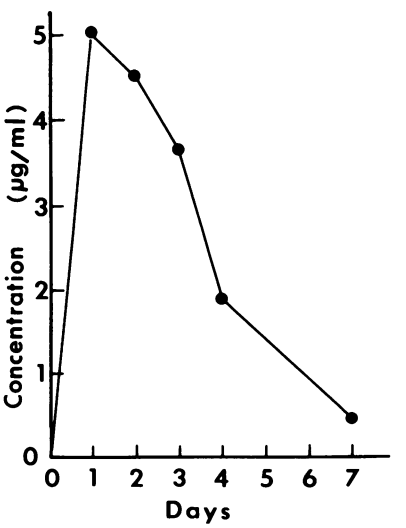

FIG. 2. *Production of formaldehyde during the anaerobic degradation of RDX.*

Hydrazines. The presence of hydrazine, 1,1-dimethylhydrazine, and 1,2-dimethylhydrazine was confirmed by GC/MS analysis of the hydrazine derivatives. The bases were isolated as their hydrochloride salts and were then treated with salicylaldehyde or with acetic anhydride to yield either the disalicyl derivative of hydrazine (salicylazine) or the acetyl derivatives of the substituted hydrazines. Infrared and GC/MS analysis and comparison with authentic salicylazine confirmed the presence of hydrazine in the reaction mixture. Treatment of the salts of the bases with acetic anhydride yielded acetyl derivatives of the dimethylhydrazines. Comparisons with authentic compounds confirmed the presence of both 1,2-dimethylhydrazine and 1,1-dimethylhydrazine.

DISCUSSION

The biodegradation of RDX occurs only under anaerobic conditions. Concurrent with the disappearance of RDX is the sequential buildup and disappearance of the mono-, di-, and trinitroso analogs of RDX (Fig. 1). The fact that HCHO formation reaches a maximum in a short time (Fig. 2), whereas that of the nitroso derivatives lags behind, tends to rule out the possibility that HCHO is produced solely by reactions subsequent to the formation of the trinitroso derivative and suggests that formaldehyde is produced early in the reaction sequence from precursors of trinitroso-RDX. Uninoculated controls containing RDX or TNX and incubated for the same periods of time as the inoculated flasks produced no HCHO. From the accumulated data we propose a pathway for the biodegradation of RDX as illustrated in Fig. 3.

In this scheme RDX is reduced sequentially to the nitroso derivatives, 2 (MNX), 3 (DNX), and 4 (TNX), each of which may undergo further reduction of a nitroso group to form the hypothetical compounds 5 (1-hydroxylamino-3,5-dinitro-1,3,5-triazine), 6 (1-hydroxylamino-3-nitroso-5-nitro-1,3,5-triazine), and 7 (1-hydroxylamino-3,5-dinitroso-1,3,5-triazine). We postulate that the molecule becomes unstable when any one of the nitro groups is reduced beyond the nitroso level. At this point hydrolytic cleavage, followed by rearrangement and further reductions of the fragments, gives rise to the end products observed. Cleavage of 5 via one route yields products 8 (*N*-hydroxymethyl-methylenedinitramine) and 9 (*N*-hydroxymethylenehydrazone), and cleavage of 6 via another route yields 10 (*N*-hydroxylamino-*N'*-nitromethylenediamine) and 11 (dimethylnitrosamine). Compound 7 undergoes cleavage via either route.

Figure 4 shows the postulated reactions of the fragments arising from the initial cleavage reaction. Cleavage of 8 releases 12 (HCHO) and 13 (methylenedinitramine), which decomposes to yield HCHO and 14 (nitramide), which in turn is reduced to 15 (hydrazine). Compound 9 rear-

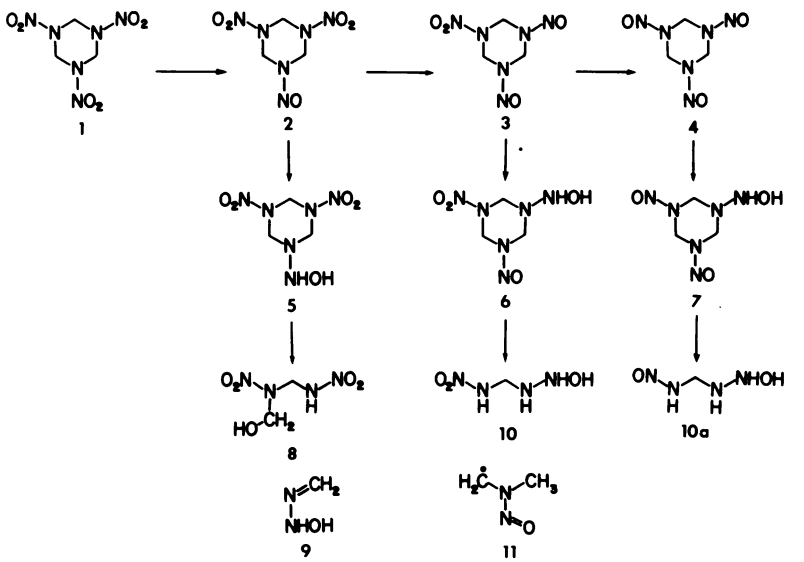

FIG. 3. *Proposed pathway for the anaerobic biodegradation of RDX. Compounds: 1, RDX; 2, MNX; 3, DNX; 4, TNX; 5, 1-hydroxylamino-3,5-dinitro-1,3,5-triazine; 6, 1-hydroxylamino-3-nitroso-5-nitro-1,3,5-triazine; 7, 1-hydroxylamino-3,5-dinitroso-1,3,5-triazine; 8, N-hydroxymethylmethylenedinitramine; 11, dimethylnitrosamine radical.*

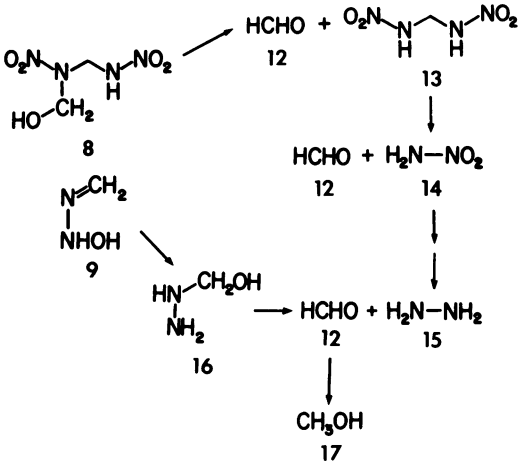

FIG. 4. *Proposed pathway for RDX biodegradation (continued). Compounds: 12, HCHO; 13, methylenedinitramine; 14, nitramide; 15, hydrazine; 16, hydroxymethylhydrazine; 17, methanol.*

ranges and is reduced to 16 (hydroxymethylhydrazine), which yields HCHO and hydrazine. Under the strongly reducing conditions formaldehyde is reduced to methanol.

As Fig. 3 shows, the other cleavage reaction (via compounds 6 and 7) yields derivatives of methylenediamine, 10 and 10a (*N*-hydroxylamino-*N'*-nitroso-methylenediamine), which follow the pathway taken by 13. As shown in Fig. 5, the other product of this cleavage, compound 11 (dimethylnitrosamine radical), either undergoes sequential reduction to 19 (1,1-dimethylhydrazine) via 18 (dimethylnitrosamine) or rearranges via 20 (hypothetical intermediate) to yield 21 (dimethyldiazene-1-oxide radical), which is reduced to 23 (1,2-dimethylhydrazine) via 22 (dimethyldiazine-1-oxide). This scheme allows for the formation of all of the observed products.

Figure 2 represents a steady-state concentration, reflecting the formation of HCHO and its concomitant reduction to methanol, presumably as soon as it is formed; thus the time of maximum net generation of HCHO may not be truly represented. If all the carbon in a solution of 50 μg of RDX per ml (225 μM) were converted to HCHO, a concentration of 676 μM HCHO (20.3 μg/ml) would result. Complete reduction to methanol would yield a methanol concentration of 21.6 μg/ml. The maximum steady-state concentration of HCHO indicated in Fig. 2 was 5 μg/ml; thus, even under steady-state conditions at least 25% of the carbon is accounted for. During the vacuum distillation of 100 ml of CMF, approximately 5 ml of distillate was collected. If 100% efficiency were attained for the quantitative distillation of methanol, then a 20-fold concentration was achieved. Since as much as 300 μg of methanol per ml was detected in one distillate sample, the original sample contained 15 μg of methanol per ml, which is consistent with the above. Thus, even in the absence of quantitative kinetic information on the formation of methanol or the hydrazines, an appreciable portion of the carbon can be accounted for. Since some of the carbon must be found in the methyl groups of the dimethylhydrazines, it is likely that we will be able to account for the majority of the carbon.

Several facts emerge pertaining to the nature of the final products remaining in solution: (i) no spargeable, volatile ^{14}C-containing compounds were detected; (ii) 98% of the radioactivity remained in the supernatant after the disappearance of RDX, formaldehyde, and the nitroso derivatives of RDX; (iii) ^{14}C was ether extractable from acidic, alkaline, or neutral solutions; and (iv) ^{14}C disappeared when either acidic, alkaline, or neutral solutions were evaporated to dryness. These findings strongly suggested the presence of carbon-containing, low-molecular-weight, polar, neutral compounds. Methanol and formaldehyde were subsequently identified as two compounds with these properties. The proposed pathway includes several additional compounds that fit this description, namely, dimethylnitrosamine and 1,2-dimethyldiazine-1-oxide (azoxymethane) (compounds 18 and 22, respectively); however, we have not detected these compounds in reaction mixtures.

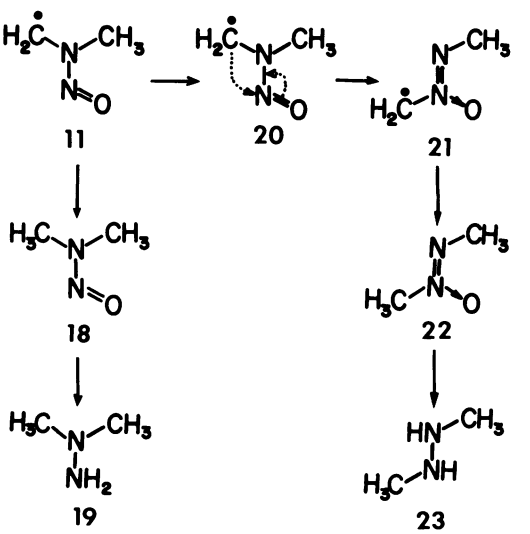

FIG. 5. *Proposed pathway for RDX biodegradation (continued). Compounds: 18, dimethylnitrosamine; 19, 1,1-dimethylhydrazine; 20, hypothetical intermediate; 21, dimethyldiazene-1-oxide radical; 22, dimethyldiazene-1-oxide; 23, 1,2-dimethylhydrazine.*

The biological treatment of RDX-containing wastes must include an anaerobic mode since no reaction occurs aerobically. Since munitions wastes generally contain high levels of nitrate, RDX wastes can be treated in an anaerobic denitrification mode. The result of such an operation would be a mixture of reduced compounds, including the hydrazines and methanol. Upon subsequent exposure to an aerobic stage, methanol would be oxidized to CO_2. Insufficient information is available on the biological fate of hydrazine and the dimethyl hydrazines. Both 1,1- and 1,2-dimethylhydrazine and their immediate precursors, dimethylnitrosamine and azoxymethane, as well as hydrazine, are known mutagens or carcinogens or both (5, 10, 21), and therefore it is most important to determine the biodegradability of these compounds. Such studies are currently in progress.

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