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# Sorption and Microbial Degradation of Toluenediamines and Methylenedianiline in Soil under Aerobic and Anaerobic Conditions

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Three of the major diamines, 2,4-toluenediamine (2,4-TDA), 2,6-toluenediamine (2,6-TDA), and 4,4′-methylenedianiline (4,4′-MDA), used as intermediates in the production of polyurethanes have been studied for their fate in soil. Previous literature has reported variable biodegradation of these industrially important compounds with no information on their expected fate in soil. Their sorption to two soils and biodegradation in soil under both aerobic and anaerobic conditions have been studied. Under aerobic and anaerobic conditions, sorption constants, $K_{oc}$, for both isomers of TDA on loam soils were 500−1300 after 8 h of contact, and the corresponding $K_{oc}$ values for MDA were 3800−5700 after 8 h of contact. Both isomers of TDA and 4,4′-MDA appear to be sorbed only a little more strongly under aerobic than anaerobic conditions. The $^{14}$carbon-labeled TDA isomers and MDA started to biodegrade immediately after mixing with aerobic soil with the recovery of 2−3% $^{14}CO_2$ after only 3 days. The biodegradation slowed later with recovery of 11−14% $^{14}CO_2$ after 28 days and an apparent 34−40% biodegradation after 1 year, based on loss of $^{14}C$. Under anaerobic methanogenic conditions, no $^{14}CH_4$ or $^{14}CO_2$ was recovered from any of the diamines after 71 days of incubation.

## Introduction

Toluenediamine (TDA) and methylenedianiline (MDA) are manufactured primarily to produce diisocyanates for use in polyurethanes. World production of TDA, usually made as an 80/20 mixture of the 2,4- and 2,6-isomers, is approximately 1 billion ($1 \times 10^9$) kg. World production of MDA is about 1.3 billion kg. MDA is often made as a mixture of about 50% 4,4′-MDA with a small amount of 2,4′-MDA and the remainder oligomers. TDA and MDA are manufactured, transported, and used in large volume with the possibility of their introduction to soil. This project was initiated to enable prediction of the fate of TDA and MDA in the soil environment, where both aerobic and anaerobic processes are important.

The only information on the sorption of TDA to soil was a report by Ode (*1*) that a laboratory lysimeter containing TDA-contaminated soil showed no TDA in leachate after 85 weeks of simulated rainfall. The biodegradation of TDA and MDA has been studied for assessment of their fate both in the environment and in connection with wastewater treatment for production facilities using a variety of test systems. Apparently, neither 2,4- nor 2,6-TDA were biodegraded as the sole carbon sources as shown in several tests for ready biodegradability (*2*−*7*). However, a variety of studies in activated sludge have shown extensive biodegradation, especially after acclimation and in the presence of cosubstrates (*8*−*12*). In a monitoring study, Snyder (*13*) reported an average of 96.4% removal of 2,4-TDA and 93.5% removal of 2,6-TDA in an industrial wastewater treatment plant (WWTP) with up to 20 mg/L TDA in the influent. Matsui et al. (*14, 15*) reported the treatment of 2,4-TDA using sludge from an operating WWTP at a large industrial complex. Nelson et al. (*16*) reported that TDA mixed with methanol and anilines in production plant still bottoms was effectively biodegraded by immobilized microbial populations in packed bed reactors.

The existing information on biodegradation of MDA is limited and inconsistent. MDA was reported not to be readily biodegradable (*2, 4, 17*). Tests for the inherent biodegradability of MDA indicate that it is biodegradable (*6, 18*). Ciba-Geigy (*17*) reported that there was only 6.5% removal of MDA in the Coupled Units Test, OECD 303 A, after 25 days of acclimation and 34 days of operation. The Coupled Units Test is a wastewater treatment simulation for determination of ultimate biodegradability described by Fischer et al. (*19*). A bench-scale biological reactor treating a mixture of amino and nitro aromatics reduced the concentration of MDA from 20 mg/L to less than 1 mg/L (*12*). Asakura et al. (*10*) reported the rapid biodegradation of MDA in the presence of aniline, and they reported little reaction in the absence of aniline.

The only reported information on the anaerobic biodegradation of either TDA or MDA is that of Krumholz et al. (*20*), who observed slow degradation of 2,4-TDA and 2,6-TDA under nitrate-reducing conditions but not under methanogenic or sulfate-reducing conditions.

## Materials and Methods

**Soil Samples.** Washington silt loam surface soil was obtained from a farm field planted with a rotation of corn and soybeans near Bethlehem, PA. Freehold sandy loam surface soil was collected from the Rutgers University Experimental Farm, in Adelphia, NJ. Both of these sites are remote from any industrial activity. The soils were partially air-dried, then passed through a 2-mm sieve, and stored for 0−9 days in plastic bags at 4 °C until used in biodegradation tests. Sterile soil subsamples for aerobic isotherm testing were prepared by autoclaving the sieved soil for 1 h each day, over 3 days, and then storing at 4 °C for 0−57 days until used. Soil properties are given in Table 1.

**Test Compounds.** Unlabeled 2,4-and 2,6-TDA were obtained in the free amine form from the Air Products and Chemicals Corporate R&D facilities (Allentown, PA). Unlabeled 4,4′-MDA (97% purity) was obtained from Aldrich Chemical Co. (Milwaukee, WI). Uniformly $^{14}C$ aromatic ring-labeled 2,4-TDA·2HCl (1.9 mCi/mmol) and 2,6-TDA·2HCl (1.7 mCi/mmol) were obtained from DuPont NEN (Boston, MA), with radiochemical purities of 99% and 94.8%, respectively, as determined by thin-layer chromatography (TLC). Uniformly $^{14}C$ aromatic ring-labeled 4,4′-MDA (free amine form; 98% purity by TLC; 15.2 $\mu$Ci/mg) was synthesized by

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**TABLE 1. Summary of Test Soil Characteristics**

| soil type | % sand | % silt | % clay | CEC[a] (mequiv/ 100 g) | pH | TOC (%) | field capacity at 1/3 bar (%) |
|---|---|---|---|---|---|---|---|
| Washington silt loam | 21 | 60 | 19 | 13.6 | 5 | 1.3 | 23.05 |
| Freehold sandy loam | 53 | 40 | 7 | 17.7 | 5.8 | 1.6 | 31.46 |

[a] CEC, cation exchange capacity.

Amersham Life Science (Arlington Heights, IL). Stock solutions of the TDAs were prepared in either acetone or water, while the MDA stock solutions were prepared in acetone. Both [1-$^{14}$C]- and [2-$^{14}$C]acetic acid, sodium salt, were obtained from DuPont NEN (Boston, MA).

**Aerobic Isotherms.** Complete isotherms were determined with autoclaved test soils in 0.01 M $CaCl_2$ in Erlenmeyer flasks shaken for 8 h. Isotherm sets were prepared with two 250-mL flasks containing either 2 g of soil and 35 mL of solution or 5 g of soil and 35 mL of solution (for 2,4- and 2,6-TDA on sandy loam) at each of six different levels of compound concentration and known $^{14}$C specific activity, using standard mixtures containing unlabeled and $^{14}$C-labeled TDA or MDA solutions. Calcium chloride solution (0.1 M) was added to each flask plus water to give a constant soil/water ratio with 0.01 M $CaCl_2$ and a range of diamine concentrations from 0.5 to 53 mg/L. In addition, duplicate flasks at the highest concentration of the series (26.2−40.2 mg/L) were set up without soil as controls, and two additional sets of duplicate flasks at an intermediate concentration (8.8−13.8 mg/L) were set up to be shaken for 7 days, counted, and then used for desorption testing (see below). A contact period of 7 days was chosen to simulate environmental contamination and to allow time for reaction with soil humic materials. All flasks were closed with rubber stoppers and shaken for either 8 h (isotherms) or 7 days at 23 °C. Preliminary studies with frequent initial sampling extending out to 7 days (data not included here) indicated that the rapid portions of the adsorption vs time curves were over by the 8-h sampling period, as only 2−11% reductions in soluble concentrations occurred between 4 and 8 h, with slower sorption rates after 8 h out to 7 days.

After being shaken, the flask contents were centrifuged in 40-mL screwcap vials at 2000 rpm for 15 min in a benchtop centrifuge, and the supernatant solutions were filtered through 0.2-$\mu$m nylon syringe filters. Filtrate (5 mL) was added to 10 mL of Beckman Ready Gel for liquid scintillation counting (LSC) of the $^{14}$C remaining in solution. Freundlich plots were constructed and a linear regression used to compute the Freundlich sorption distribution coefficient, $K_d$, and the slope ($1/n$). The Freundlich distribution coefficient was normalized to the fraction ($f_{oc}$) of organic carbon in the soil (0.01 × % TOC in Table 1) by division ($K_d/f_{oc} = K_{oc}$). Distribution ratios ($R_{sorb}$) were calculated from data collected from duplicate flasks at a single, intermediate concentration (8.8−13.8 mg/L) after 7 days:

$$R_{sorb} = (\mu g/g \text{ on soil})/(\mu g/mL \text{ of concentration in solution})$$

**24-h Aerobic Soil Desorption Tests.** The duplicate sets of flasks that were shaken for 7 days were processed as described above for the 8-h isotherm flasks. All of the supernatant solution was then removed and replaced with sufficient fresh 0.1 M $CaCl_2$ and deionized water to give the original solution phase volume and a 0.01 M concentration of $CaCl_2$. The soil pellet was resuspended, and the soil suspension was shaken for 24 h at 23 °C. After being shaken, the flask was processed as described above, and the distribution ratios for desorption ($R_{desorb}$) were calculated from the solution concentration of compound and the calculated diamine remaining on the soil after 24 h of desorption:

$$R_{desorb} = (\mu g/g \text{ on soil})/(\mu g/mL \text{ of concentration in solution})$$

**Anaerobic Isotherms.** To examine the potential effect of oxidative reactions on the soil adsorption of TDA and MDA, isotherms were determined under anaerobic conditions. Two grams of 2-mm sieved silt or sandy loam soils were weighed into acid-washed, heat-sterilized glass 40-ml vials with hole-type plastic screwcaps and Teflon-lined rubber septa. Next, helium-stripped deionized water and helium-stripped 0.1 M $CaCl_2$ solution plus sodium acetate and glucose solutions (stripped with 30% $CO_2$/70% $N_2$) were added. The total solution volume was 35 mL minus the volumes of TDA or MDA solutions, which were to be added later after the soils had attained anaerobic conditions (final isotherm concentrations were 0.01 M $CaCl_2$ and 0.0125 M acetate and glucose). Two additional vials containing soils, $CaCl_2$, acetate, and glucose solutions were also set up to be opened as surrogates for the other vials to determine oxidation−reduction potential (ORP) conditions prior to the additions of TDA and MDA. All of the vials were purged for 4 min with 50 cm$^3$/min of a 30% $CO_2$/70% $N_2$ gas mixture to remove dissolved oxygen and then were capped and allowed to stand in darkness for 3−5 days to allow development of anaerobic conditions.

After incubation, the two anaerobic surrogate vials were opened inside an $N_2$ atmosphere glovebag to determine their ORP, using an Orion combination platinum wire/reference cell electrode and a pH meter set for millivolt readout. The electrode was checked with ZoBell's solution (*21*). When the ORP in the surrogate vials was −50 mV or less, the other vials in the test set were placed into the glovebag and injected through their septa with a (constant) volume of aqueous $^{14}$C-labeled TDA or MDA solution plus various volumes of nonlabeled aqueous TDA or MDA solutions, respectively.

Isotherms were run with six concentration levels of the nonradioactive compound plus a known (constant) concentration of $^{14}$C-labeled TDA or MDA, set up in duplicate with concentrations of 0.3−40 mg/L diamine. In addition, duplicate control vials were set up with the $^{14}$C -labeled and nonlabeled compound, but without soil, at the highest concentration of the series (26.2−40.2 mg/L); an additional set of duplicate vials at an intermediate compound concentration (8.8−13.8 mg/L) was set up to be shaken for 7 days and then used for desorption testing (see below). After being spiked, the vials were shaken on a Burrell wrist action shaker at 23 °C for either 8 h (isotherms) or 7 days.

After being shaken, the vials were centrifuged and then filtered through 0.2 $\mu$m nylon syringe filters inside an $N_2$ atmosphere glovebag for LSC counting of 5 mL of the supernatant solutions. Freundlich distribution coefficients ($K_d$ and $K_{oc}$) and 7-day sorption distribution ratios ($R_{sorb}$) were calculated as described above for aerobic isotherms.

**24-h Anaerobic Soil Desorption Tests.** The duplicate vials that were shaken for 7 days were processed as described above for the aerobic desorption. After the 24-h desorption period, the distribution ratios for desorption ($R_{desorb}$) were calculated as given above.

**Aerobic Biodegradation Tests.** The test flasks were similar to those described by Gledhill (*22*) and were constructed from 500-mL Erlenmeyer flasks with 24/40 ground-glass joints. The inner test tube section had gas vent openings and contained 4 mL of 1 N NaOH solution to trap respired $^{14}CO_2$. A Teflon 24/40 sleeve was placed over the ground-glass joint of the trap tube, the joint was clamped with an ACE Glass Catalog No. 7598 24/40 size plastic clamp, and a Teflon screw thread plug with an O-ring (Ace Catalog No. 5845-45) was used to seal the top of the tube. This plug allowed sampling of the NaOH solution for LSC analysis of trapped $^{14}CO_2$.

A sample of 50 g of air-dried soil, prepared as described above, was weighed into each test flask. The volume of added water needed to provide 75% of 1/3 bar moisture tension in the soil (Table 1) was calculated from the moisture analysis. This volume was divided between water and aqueous spike solutions of $^{14}$C-labeled test compounds. The compounds were added to the soil at the 5 $\mu$g/g (ppm) level and at approximately 4−5 $\times 10^6$ DPM of $^{14}$C per flask (1.8−2.3 $\mu$Ci per flask). The test substance concentration was chosen to avoid any inhibition of biological activity and to facilitate reliable analyses. Duplicate flasks were set up for each TDA or MDA compound tested and for each incubation time period to be tested. One control flask (no added radiolabeled test compound) was set up for each incubation time period. NaOH solution was added to each $^{14}CO_2$ trap tube, and the sealed flasks were incubated in darkness at 25 °C for periods up to 1 year. At the end of each incubation period, the duplicate sets of flasks were sampled for the cumulative trapped $^{14}CO_2$. The flasks were sacrificed to provide soil for a water extraction test and for thermal oxidation analysis of the bound residual soil $^{14}$C, as described below.

At each sampling time, the test tube section of the flask was lifted from the vial, and the soil in the flasks was wetted with 20 mL of 0.01 N NaOH and then quickly acidified with 10 mL of 1:1 $H_3PO_4$. The test tube section was then immediately replaced on the flask, and the sealed flasks were allowed to stand for 24 h. The NaOH in the $^{14}CO_2$ trap was removed for LSC counting with Beckman Ready Gel liquid scintillation cocktail and then replaced with a second 4-mL portion of fresh 1 N NaOH, and the flask was allowed to stand for a second 24-h period to complete the $^{14}CO_2$ absorption in the NaOH. Liquid scintillation counting (LSC) was performed on a Beckman Model LS3801 instrument with quench correction provided by an external standard of $^{137}$cesium. The $^{14}$C activity values in the two NaOH traps were summed to get the percent conversion of aromatic ring-labeled $^{14}$C in the TDA or MDA to $^{14}CO_2$.

**Residual Water-Extractable $^{14}$C.** The soil remaining in the respirometer flask was mixed with an additional 70 mL of deionized water, and the flask was stoppered and shaken for 24 h on a wrist shaker. The soil suspensions were then centrifuged, and the supernatant solutions were decanted and filtered through 0.2 $\mu$m pore size nylon syringe filters; then 5 mL of filtrate was counted with 10 mL of Beckman Ready Gel.

**Bound Residual Soil $^{14}$C.** The centrifuged soil left from the water extraction was allowed to air-dry and then was pulverized, and duplicate 1-g samples were weighed into ceramic sample boats along with 5 mg of cellulose powder and oxidized for 4 min to form $^{14}CO_2$ from all forms of residual $^{14}$C in a Harvey Instruments Model OX-500 sample oxidizer tube furnace at 750 °C in the combustion zone and 680 °C in the catalyst zone. The $^{14}CO_2$ gas exiting the furnace was trapped by passage through 15 mL of Harvey $^{14}$C liquid scintillation cocktail in each of two LSC vials placed in series, downstream of the furnace. The two solutions were then counted for $^{14}$C activity by LSC and summed to obtain the activity of residual $^{14}$C in the soil.

**Anaerobic Biodegradation Tests in Soil.** Fresh air-dried, sieved silt loam (20 g) was weighed into acid-washed, dried 160-mL serum bottles. Helium-stripped, deionized water plus 8 mL of 0.1 M $CaCl_2$ plus 2 mL of 0.1 M sodium acetate and 2.1 mL of 0.1 M glucose solution (both purged with a gas mixture of 30% $CO_2$/70% $N_2$ to remove $O_2$) were added to give a final volume of 80 mL minus the volumes of [$^{14}$C]TDA, [$^{14}$C]MDA, or [2-$^{14}$C] sodium acetate to be added after the bottles had attained an anaerobic condition. All test conditions were set up in duplicate bottles. Surrogate bottles were also set up with the soil, $CaCl_2$, and glucose/acetate nutrients to be opened for determining the oxidation−reduction potential (ORP) of the bottles.

The contents of the bottles were stripped with 50 mL/min of $CO_2/N_2$ gas mixture for 4 min and then stoppered with gray butyl rubber caps sealed with aluminum-crimped seals. They were then incubated at 30 °C for 6−8 days to allow the bottles to develop anaerobic conditions before adding test compounds. After incubation, two surrogate bottles were opened inside an $N_2$ atmosphere glovebag to determine the ORP, as described above. If the ORP in the surrogate bottles was at −50 mV or less, the other bottles in the test set were placed into the glovebag, and the bottles were spiked with $^{14}$C-labeled compounds via syringe injection through the rubber cap. Duplicate bottles were set up to be analyzed for $^{14}CO_2$ and $^{14}CH_4$ gases after 0, 33, 45, and 73 days at 30 °C.

**Anaerobic Biodegradation Tests in Soil + Anaerobic Sewage Sludge.** A set of bottles was also set up with soil + anaerobic digester sludge from the Lehigh County Sewage Pretreatment Plant in Trexlertown, PA. The sludge solids were passed through a 2-mm sieve and then diluted by 1/5 with a sterile nutrient medium described by ASTM (*23*), but made up at 2 times the normal nutrient medium strength.

This sludge mixture (40 mL) was added to each bottle in addition to the 40 mL total volume of soil/water. These bottles were handled as described above. Surrogate bottles containing soils and soils + sludge but with no labeled substrates were also set up to be opened for determining ORP conditions prior to spiking. Sludge/soil bottles were incubated for 0, 33, 45, and 71 days at 30 °C and analyzed in the same manner as the bottles containing only soil.

**Analysis of $^{14}CO_2$ and $^{14}CH_4$ in Anaerobic Test Bottles.** The setup to measure $^{14}CO_2$ and $^{14}CH_4$ was similar to that described by Steber and Wierich (*24*) and Nuck and Federle (*25*), except that the serum bottles were sealed until sacrificed for analysis. A nitrogen purge gas stream was first passed through the serum bottle and then through two 40-mL vial traps in series, each containing 35 mL of 0.8 M NaOH. The $^{14}CO_2$ gas was trapped in NaOH, and $^{14}CH_4$ gas was allowed to pass through due to the high distribution constant for $CH_4$ between gas and water phases. The outlet of the second NaOH trap was connected to the inlet of a Harvey Model OX-500 biological oxidizer tube furnace to convert all $^{14}CH_4$ gas to $^{14}CO_2$. This $^{14}CO_2$ from methane oxidation in the furnace was trapped in two vials connected in series, each containing 15 mL of R. J. Harvey carbon 14 cocktail. The $O_2$ and $N_2$ flows to the OX-500 furnace were both reduced to 250 cm$^3$/min instead of their normal values of 350 cm$^3$/min, and the added $N_2$ purge gas flow through the serum bottle and NaOH trap vials was 100 cm$^3$/min. This analysis system was tested by analysis of $NaH^{14}CO_3$, in serum bottles with averages of 93.7% caught in the NaOH traps and only 0.7% passed through into the Harvey traps. In another test, [$^{14}$C] toluene was purged from the serum bottle through the NaOH traps and into the oxidizer with an average of 94.8% recovery in the Harvey traps.

For analysis, the serum bottles were initially connected to the oxidation/trap system to relieve any excess gas pressure in the bottles and to trap/oxidize excess $^{14}CO_2$ or $^{14}CH_4$ gas. Then 1.4 mL of 1:1 $H_3PO_4$ was injected through the cap of the bottle in order to convert all of the [$^{14}$C] carbonate and [$^{14}$C]bicarbonate in the serum bottle to gaseous $^{14}CO_2$, and the purge gas flow was started through the bottle via a long 1/16-in. stainless steel tube passed through the rubber cap. The serum bottle purge flow was continued for four 4-min

TABLE 2. Calculated Freundlich Distribution Coefficients ($K_d$), Slopes ($1/n$), and Coefficients of Determination ($r^2$) from 8-h Isotherms

| | sandy loam | | | | | | silt loam | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | aerobic | | | anaerobic | | | aerobic | | | anaerobic | | |
| compd | $K_d$ | $1/n$ | $r^2$ | $K_d$ | $1/n$ | $r^2$ | $K_d$ | $1/n$ | $r^2$ | $K_d$ | $1/n$ | $r^2$ |
| 2,4-TDA | 21.3 | 0.6 | 0.9993 | 11.4 | 0.74 | 0.9922 | 17.5 | 0.57 | 0.9998 | 13.7 | 0.70 | 0.9948 |
| 2,6-TDA | 11.3 | 0.6 | 0.9960 | 9.4 | 0.70 | 0.9975 | 10.5 | 0.57 | 0.9973 | 7.1 | 0.70 | 0.9898 |
| 4,4′-MDA | 90.9 | 0.7 | 0.9945 | 61.2 | 0.74 | 0.9668 | 52.2 | 0.67 | 0.9991 | 49.8 | 0.68 | 0.9911 |

TABLE 3. Calculated $K_{oc}$ Sorption Coefficients from 8-h Isotherms

| | $K_{oc}$ in sandy loam | | $K_{oc}$ in silt loam | |
|---|---|---|---|---|
| compd | aerobic | anaerobic | aerobic | anaerobic |
| 2,4-TDA | 1331 | 713 | 1346 | 1054 |
| 2,6-TDA | 706 | 588 | 808 | 546 |
| 4,4′-MDA | 5681 | 3825 | 4015 | 3831 |

TABLE 4. Calculated Distribution Ratios from Sorption and Desorption after 7 Days of Sorption Followed by 1 Day of Desorption

| | aerobic | | | |
|---|---|---|---|---|
| | sandy loam | | silt loam | |
| compd | $R_{sorb}$ | $R_{desorb}$ | $R_{sorb}$ | $R_{desorb}$ |
| 2,4-TDA | 240 | 583 | 170 | 464 |
| 2,6-TDA | 102 | 373 | 196 | 598 |
| 4,4′-MDA | 101 | 173 | 55 | 103 |

| | anaerobic | | | |
|---|---|---|---|---|
| | sandy loam | | silt loam | |
| compd | $R_{sorb}$ | $R_{desorb}$ | $R_{sorb}$ | $R_{desorb}$ |
| 2,4-TDA | 47 | 188 | 54 | 263 |
| 2,6-TDA | 26 | 115 | 20 | 145 |
| 4,4′-MDA | 186 | 234 | 126 | 151 |

| | ratio $R_{desor}/R_{sorb}$ | | | | | |
|---|---|---|---|---|---|---|
| | aerobic | | | anaerobic | | |
| compd | sandy loam | silt loam | av | sandy loam | silt loam | av |
| 2,4-TDA | 2.4 | 2.7 | 2.6 | 4.0 | 4.8 | 4.4 |
| 2,6-TDA | 3.7 | 3.0 | 3.3 | 4.4 | 7.1 | 5.8 |
| 4,4′-MDA | 1.7 | 1.9 | 1.8 | 1.3 | 1.2 | 1.2 |

consecutive oxidation cycles of the OX-500. The sum of the $^{14}C$ activities in the two NaOH traps was used to calculate the $^{14}CO_2$ production, and the sum of the activities in the two Harvey LSC vials was used for the $^{14}CH_4$ production.

**Soluble $^{14}C$ Remaining.** After the gas analysis, the serum bottle was removed from the apparatus, and the solids were allowed to settle. An aliquot of the supernatant solution was filtered through a 0.2-μm nylon syringe filter for LSC counting of the soluble $^{14}C$.

**Bound Residual Soil $^{14}C$.** The supernatant solution not used for counting was decanted off the settled soil or soil/sludge suspension, and the wet solids were air-dried for combustion analysis on the Harvey OX-500, as described above.

## Results and Discussion

**Aerobic and Anaerobic Sorption.** Soil sorption of the $^{14}C$-labeled 4,4′-MDA was rapid under both aerobic and anaerobic conditions in the two types of soils tested. The Freundlich distribution coefficients ($K_d$), slopes, $1/n$, and Microsoft Excel spreadsheet LINEST function coefficient of determination ($r^2$) measured after 8 h of equilibration time, are given in Table 2. Comparisons of 8-h and 7-day sorption periods had shown that, for MDA, the 8-h values were 92% of the 7-day values for both soils. Preliminary experiments had shown that most of the sorption occurred within 4 h. The sorption of MDA to soil was normalized to the soil organic carbon content to give the sorption constant, $K_{oc}$, in Table 3. This makes it possible to compare chemicals in different soils more easily. MDA was strongly sorbed, with $K_{oc}$ values of several thousand.

The soil sorption of 2,4-TDA and 2,6-TDA was initially rapid. The $K_d$, $K_{oc}$, $1/n$, and $r^2$ values measured after 8 h of equilibration time are given in Tables 2 and 3. At 8 h, the TDA $K_{oc}$ values were greater than 500. McCall et al. (*26*) classified chemicals for their soil mobility potential according to their $K_{oc}$ values. The observed $K_{oc}$ values for TDA place it in the "low" mobility class at 8 h. The observed $K_{oc}$ values for MDA correspond to "slight" in this classification.

The potential for slower, irreversible reactions of TDA and MDA with soil humics was studied by allowing the compounds to remain in contact with soil for 7 days and then measuring desorption in 1 day. The distribution ratios of TDA and MDA with soil after 7 days of sorption and 1 day of desorption are presented in Table 4. These results suggest that MDA sorbed on soil may be in a relatively facile equilibrium with the surrounding solution as shown by the similarity of distribution ratios [sorbed compound (in μg/g of soil)/concentrated in solution (in μg/mL)] for both sorption and desorption, with $R_{desorb}/R_{sorb}$ ranging from 1.1 to 1.4. In contrast, both isomers of TDA desorbed to a much smaller extent, $R_{desorb}/R_{sorb}$ ranging from 3.0 to 4.6. This is consistent with the formation of complexes by TDA with the humic materials or other irreversible soil binding process.

Reactions of aromatic amines with soil humics have been suggested by a number of researchers, principally Bartha and co-workers (*27*−*30*) and Bollag and co-workers (*31*−*37*). The reactions reported by Bollag and co-workers between aniline compounds and humics are primarily oxidative. Kosson and Byrne (*38*) reported the apparent release of bound, nonextractable aniline from soil under both denitrifying and methanogenic anaerobic conditions. If soil oxidative reactions were important for the diamines in this study, a significant difference between the behavior of diamines with soil under oxidizing and reducing conditions would be expected. In this study, the average ratio of aerobic/anaerobic soil sorption coefficients ($K_{oc}$) for 2,4-TDA, 2,6-TDA, and 4,4′-MDA were 1.57, 1.34, and 1.27, respectively. Both isomers of TDA and 4,4′-MDA appear to be sorbed only a little more strongly under aerobic than anaerobic conditions.

**Biodegradation.** Table 5 shows the distribution of $^{14}C$ in preliminary aerobic biodegradation experiments, and Table 6 shows the distribution of $^{14}C$ out to 1 year of incubation. The extent of biodegradation can be taken as either the fraction of $^{14}CO_2$ recovered or as $^{14}C$ lost from the soil and solution. The amount of $^{14}CO_2$ recovered peaked at 56 days, and a bit less was recovered at 210 and 365 days, indicating

**TABLE 5. Short-Term Aerobic Biodegradation of 2,4-TDA 2,6-TDA, and MDA in Silt Loam Soil[a]**

| | | compounds spiked | | |
|---|---|---|---|---|
| time (days) | $^{14}$C fractionation | 2,4-TDA av | 2,6-TDA av | 4,4-MDA av |
| 1 | % as $^{14}CO_2$ | 1.3 | 1.7 | 2.0 |
| 1 (autoclaved) | % as $^{14}CO_2$ | 0.0 | 0.0 | 0.0 |
| 3 | % as $^{14}CO_2$ | 1.9 | 2.7 | 2.9 |
| | % in solution | 2.1 | 0.9 | 0.5 |
| 3 (autoclaved) | % as $^{14}CO_2$ | 0.1 | 0.0 | 0.0 |
| | % in solution | 1.4 | 1.4 | 0.3 |
| 7 | % as $^{14}CO_2$ | 4.0 | 6.2 | 9.1 |
| | % in solution | 3.5 | 2.0 | 1.3 |
| 14 | % as $^{14}CO_2$ | 5.7 | 8.1 | 9.7 |
| | % in solution | 3.8 | 2.5 | 1.1 |
| | % on soil | 78.5 | 81.7 | 84.5 |
| | matl balance[b] | 88.0 | 92.3 | 95.3 |

[a] Values are averages of two test flasks. [b] Material balance = sum of % initial $^{14}C$ as $^{14}CO_2$ + $^{14}C$ in solution + residual $^{14}C$ on soil after 14 days.

**TABLE 6. Long-Term Aerobic Biodegradation of 2,4-TDA, 2,6-TDA, and MDA in Silt Loam Soil**

| | | compounds spiked | | |
|---|---|---|---|---|
| time (days) | $^{14}$C fractionation | 2,4-TDA av | 2,6-TDA av | 4,4′-MDA av |
| 0 | % as $^{14}CO_2$ | 1.0 | 2.4 | 1.6 |
| | % in solution | 4.1 | 3.5 | 3.0 |
| | % on soil | 91.0 | 84.6 | 91.8 |
| | matl balance[a] | 96.1 | 90.4 | 96.4 |
| 14 | % as $^{14}CO_2$ | 7.9 | 11.1 | 10.0 |
| | % in solution | 5.4 | 2.7 | 1.5 |
| | % on soil | 78.5 | 81.7 | 84.5 |
| | matl balance | 91.8 | 95.4 | 96.0 |
| 28 | % as $^{14}CO_2$ | 10.8 | 14.2 | 11.2 |
| | % in solution | 5.8 | 2.7 | 1.4 |
| | % on soil | 84.2 | 80.3 | 89.3 |
| | matl balance | 100.7 | 97.3 | 101.9 |
| 56 | % as $^{14}CO_2$ | 14.8 | 18.1 | 11.6 |
| | % in solution | 6.3 | 3.4 | 1.6 |
| | % on soil | 86.8 | 80.5 | 91.7 |
| | matl balance | 107.8 | 101.9 | 104.9 |
| 210 | % as $^{14}CO_2$ | 15.8 | 15.9 | 9.7 |
| | % in solution | 5.8 | 3.8 | 1.7 |
| | % on soil | 57.4 | 73.9 | 67.8 |
| | matl balance | 79.0 | 93.6 | 79.2 |
| 365 | % as $^{14}CO_2$ | 14.7 | 13.8 | 8.9 |
| | % in solution | 5.8 | 3.8 | 1.7 |
| | % on soil | 60.0 | 60.5 | 58.2 |
| | matl balance | 80.5 | 78.0 | 68.7 |

[a] Material balance = sum of % initial $^{14}C$ as $^{14}CO_2$ + $^{14}C$ in solution + residual $^{14}C$ on soil after each test period.

a loss of $^{14}C$ at the latter sampling times (see below). Figure 1 shows the extent of biodegradation with time for both isomers of TDA and MDA based on $^{14}CO_2$ recovered for the first 56 days and as $^{14}C$ lost from the soil or solution thereafter. The $^{14}C$-labeled TDA isomers and MDA started to biodegrade immediately after mixing with aerobic soil with the recovery of 2 or 3% $^{14}CO_2$ after only 3 days. Autoclaved soil showed less than 0.1% $^{14}CO_2$ in 3 days, indicating that the $^{14}CO_2$ is due to biological activity. A small percentage (1−2.4%) of the initial $^{14}C$ spike was found in the NaOH trap solutions of the 0-day flasks, analyzed 4−6 h after each compound spiked. Addition of barium chloride to portions of the NaOH solutions, followed by $BaCO_3$ precipitation and LSC counting of the supernatant solutions, indicated that the trapped $^{14}C$ was >99% $^{14}CO_2$. Only 5−6% of the initial $^{14}C$ spike was found in the solution phase of the soil test flasks, indicating only a small yield of soluble metabolites.

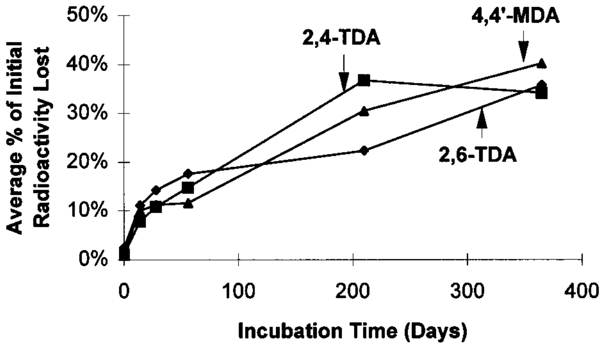

**FIGURE 1. Aerobic biodegradation of 4,4′-MDA (▲), 2,4-TDA (■), and 2,6-TDA (♦) in silt loam soil.**

The observed biodegradation of both isomers of TDA and MDA is consistent with the literature, which reports aerobic biodegradation in the presence of other degradable organics (as summarized in the Introduction). Since the silt loam soil used in this study had been cultivated for corn and soybeans, there is a possibility of past contact with metalochlor, a herbicide which contains an aromatic amine. No supplementary carbon substrate was added to the soil in this study. The amount of organics present in the soil as collected, a TOC of 1.3%, was several thousand times the concentration of added TDA or MDA. The slowing of biodegradation after 2 weeks may be related to the formation of tightly bound complexes between the diamines and soil humic materials or exhaustion of oxygen in the flask atmosphere. Slowing of the biodegradation rate of aniline compounds because of sorption to soil has been noted before by You and Bartha (*27*), Hsu and Bartha (*29*), and Kosson and Byrne (*38*). The 50 g of soil in each flask contained 0.6 g of carbon or 50 mmol of carbon. The atmosphere in the flasks contained only about 4 mmol of oxygen. Thus, extensive oxidation of the soil humics could have consumed all the oxygen and/or overloaded the NaOH traps with $CO_2$.

The material balance was significantly less at the last two sampling times as shown in Table 6. As described in the results above, this may have resulted from either loss of $^{14}CO_2$ gas or incomplete combustion of the bound residue of the diamine on the analyzed soil sample with the Harvey sample oxidizer. Balba et al. (*39*) report more than 80% release of bound 4-chloroaniline from soil by pyrolysis in helium at 550 °C. Woroby and Webster (*40*) reported that the amount of 4-chloroaniline released by pyrolysis varied depending on the chemical concentration and properties of the soil and seemed independent of the temperature at 200−900 °C. Based on this work by Woroby and Webster and the experiments in the present study, (a) varying the combustion temperature from 750 to 900 °C, (b) combustion of a $^{14}C$ standard, Spec-Chec, and (c) low residual activity in the deposits scraped out of the combustion tube, it is the opinion of the authors that the missing $^{14}C$ resulted from leakage of $^{14}CO_2$ during incubation. Therefore, the extent of biodegradation at the conclusion of the aerobic biodegradation study, 210 and 365 days, can best be represented by 100% minus the $^{14}C$ found in the soil and water solution. Table 6 has the information for these calculations as well as the $^{14}CO_2$ recovered. For example, the biodegradation of 2,4-TDA at 365 days is calculated by adding the amount of $^{14}C$ found in solution (5.8%) and that found on the soil by combustion (60.0%) of the initial $^{14}C$ and subtracting from 100% of the initial $^{14}C$ to obtain an apparent biodegradation of 34.2%. Similarly, the apparent biodegradation of 2,6-TDA and 4,4′-MDA after 365 days is calculated to be 35.7% and 40.1%, respectively.

**TABLE 7. Anaerobic Biodegradation of $^{14}$C-Labeled Compounds in Silt Loam Soil + Sewage Sludge[a]**

| time (days) | $^{14}$C fractionation | compounds spiked 2,4-TDA av | 2,6-TDA av | 4,4′-MDA av | [2-$^{14}$C]acetate av |
|---|---|---|---|---|---|
| 0 | % as $^{14}CO_2$ | 0.9 | 0.1 | 0.0 | 6.5 |
| | % as $^{14}CH_4$ | 0.0 | 0.0 | 0.0 | 27.2 |
| | total $^{14}$C gas | 0.9 | 0.1 | 0.0 | 33.7 |
| | % in solution | 42.9 | 52.1 | 11.4 | 55.1 |
| 33 | % as $^{14}CO_2$ | 0.0 | 0.0 | 0.0 | 29.2 |
| | % as $^{14}CH_4$ | 0.0 | 0.0 | 0.0 | 60.6 |
| | total $^{14}$C gas | 0.0 | 0.0 | 0.0 | 89.8 |
| | % in solution | 2.3 | 2.8 | 1.0 | 0.0 |
| 45 | % as $^{14}CO_2$ | 0.0 | 0.0 | 0.0 | 31.6 |
| | % as $^{14}CH_4$ | 0.0 | 0.0 | 0.0 | 59.5 |
| | total $^{14}$C gas | 0.0 | 0.0 | 0.0 | 91.1 |
| | % in solution | 2.1 | 2.3 | 0.9 | 0.0 |
| 71 | % as $^{14}CO_2$ | 0.0 | 0.0 | 0.0 | 29.9 |
| | % as $^{14}CH_4$ | 0.0 | 0.0 | 0.0 | 59.7 |
| | total $^{14}$C gas | 0.0 | 0.0 | 0.0 | 89.6 |
| | % in solution | 1.9 | 2.1 | 0.8 | 0.3 |
| | % on soil | 94.0 | 86.8 | 90.8 | 3.8 |
| | matl balance | 95.8 | 88.9 | 91.6 | 93.6 |

[a] Values are averages of two test flasks. [b] Material balance = sum of % initial $^{14}$C as $^{14}CO_2$ + $^{14}CH_4$ + $^{!\$}$C in solution + residual $^{14}$C on soil after 71 days.

Table 7 shows the distribution of $^{14}$C from 2,4-TDA, 2,6-TDA, 4,4′-MDA, and [2-$^{14}$C]acetate in anaerobic soil with anaerobic digester sludge. Under methanogenic conditions, no radiolabeled methane ($^{14}CH_4$) or carbon dioxide ($^{14}CO_2$) was observed from any of the three diamines after 71 days of incubation. A similar study using anaerobic soil without added sludge gave the same results, no detected $^{14}CH_4$ or $^{14}CO_2$. The [$^{14}$C]acetate-positive controls produced $^{14}CH_4$ and $^{14}CO_2$ as expected. The acetate was degraded very rapidly, especially in the bottles with the active sewage sludge, producing $^{14}CH_4$ and $^{14}CO_2$ even at the nominal zero time (4−6 h). The low amounts of $^{14}$C observed in solution in bottles spiked with diamines (1−2%) correspond approximately to the concentrations of diamine expected from the soil sorption study above.

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## Literature Cited

(1) Ode, R. H. Bayer Corp., personal communication, 1993.

(2) MITI/CITI. *Biodegradation and Bioaccumulation Data on Existing chemicals Based on CSCL*; Japan, 1993; Cited in Diaminotoluene EUCLID Data sheet, 1995.

(3) Fujiwara, K. *Studies on the Effect of TDA and MDA on Plankton-Daphnia*; Report to the International Isocyanate Institute, Project FE-E-26; University of Tsukuba: Japan, 1982.

(4) Yakabe, Y. *Biodegradability Test of 2,4-Diaminotoluene and 4,4′-Diaminodiphenylmethane*; Report to the International Isocyanate Institute, Project 105-FE-E Part III: CITI, Kurume, Japan, 1994.

(5) Bayer, AG. Unpublished work; Cited in Diaminotoluene EUCLID Data Sheet, 1995.

(6) Caspers, N.; Hamburger, B.; Kanne, R.; Klebert, W. *Ecotoxicity of Toluenediisocyanate (TDI), Diphenyl-methanediisocyanate (MDI), Toluenediamine (TDA), Diphenylmethanediamine (MDA)*; Report to the International Isocyanate Institute, Project E-CE-41; Bayer AG, 1987.

(7) Ilisescu, A.; Stefanescu, L. *Stud. Epurarea. Apelor.* **1974**, *16*, 76−98.

(8) BASF AG. unpublished work, 1993; Cited in Diaminotoluene EUCLID Data Sheet, 1995.

(9) Asakura, S.; Okazaki, S. *Chemosphere* **1995**, *30*, 2209−2217.

(10) Asakura, S. report to the International Isocyanate Institute, Project FE-E-39; Yokohama National University: Japan, 1986; Cited in 4,4′-Methylenedianiline EUCLID Data Sheet, 1995.

(11) Morris, R. L. Mobay Chemical Corp., personal communication, 1988.

(12) Sandridge, R. L. Mobay Chemical Corp. letter to M. Nakarta, 1984; Cited in Diaminotoluene EUCLID Data Sheet, 1995.

(13) Snyder, K. B. Air Products and Chemicals, Inc., personal communication, 1991.

(14) Matsui, S.; Murakami, T.; Sasaki, T.; Hirose, Y.; Iguma, Y. *Prog. Water Technol.* **1975**, *7*, 645−659.

(15) Matsui, S.; Okawa, Y.; Ota, R. *Water Sci. Technol.* **1988**, *20*, 201−210.

(16) Nelson, J. A.; Clark, M. L.; Jones, G.; Andrews, S.; Portier, R. J. Field Evaluation of a Packed Bed Immobilized Microbe Reactor for the Continuous Biodegradation of Still Bottom Wastewaters. Presented at the International Conference on Physicochemical and Biological Detoxification of Hazardous Wastes, Atlantic City, NJ, 1988.

(17) Ciba-Geigy Ltd. 1985 and 1986, unpublished reports; Cited in 4,4′-Methylenedianiline EUCLID Data Sheet, 1995.

(18) BASF AG. unpublished report, 1988; Cited in 4,4′-Methylenedianiline EUCLID Data Sheet, 1995.

(19) Fischer, W. K.; Gerike, P.; Holtmann, W. *Water Res.* **1975**, *8*, 1131−1135.

(20) Krumholz, L. R.; Li, J.; Clarkson, W. W.; Wilber, G. G.; Suflita, J. M. *J. Ind. Microbiol. Biotechnol.* **1997**, *18*, 161−169.

(21) American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federation (WEF). *Standard Methods for the Analysis of Water and Wastewater*, 18th ed.; Greenberg, A. E., Clesceri, L. S., Eaton, A. D., Eds.; APHA: New York, 1992; pp 2−62.

(22) Gledhill, W. E. *Appl. Microbiol.* **1975**, *30*, 922.

(23) Standard Test Method for Determining the Anaerobic Biodegradation Potential of Organic Compounds. *Annual Book of ASTM Standards Vol. 11.05*; 1995; E 1196−92.

(24) Steber, J.; Wierich, P. *Water Res.* **1987**, *21*, 661−667.

(25) Nuck, B. A.; Federle, T. W. *Environ. Sci. Technol.* **1996**, *30*, 3597−3603.

(26) McCall, P. J.; Swann, R. L.; Laskowski, D. A.; Unger, S. M.; Vrona, S. A.; Dishburger, H. L. *Bull. Environ. Contam. Toxicol.* **1980**, *24*, 190−195.

(27) You, I. S.; Bartha, R. *J. Agric. Food Chem.* **1982**, *30*, 274−277.

(28) Hsu, T.; Bartha, R. *Soil Sci.* **1974**, *116*, 444−452.

(29) Hsu, T.; Bartha, R. *J. Agric. Food Chem.* **1976**, *24*, 118−122.

(30) Saxena, A.; Bartha, R. *Soil Sci.* **1983**, *136*, 111−116.

(31) Bollag, J.-M.; Minard, R. D.; Liu, S.-Y. *Environ. Sci. Technol.* **1983**, *17*, 72−80.

(32) Bollag, J.-M.; Liu, S.-Y.; Deune, E. G. *Soil Sci.* **1987**, *143*, 56−65.

(33) Bollag, J.-M.; Myers, C. J.; Minard, R. D. *Sci. Total Environ.* **1992**, *123/124*, 205−217.

(34) Liu, S.-Y.; Minard, R. D.; Bollag, J.-M. *J. Environ. Qual.* **1987**, *16*, 48−53.

(35) Tatsumi, K.; Freyer, A.; Minard, R. D.; Bollag, J.-M. *Environ. Sci. Technol.* **1994**, *28*, 210−215.

(36) Bollag, J.-M.; Russel, S. *Microb. Ecol.* **1976**, *3*, 65−73.

(37) Bollag, J.-M.; Blattman, P.; Laanio, T. *J. Agric. Food Chem.* **1978**, *26*, 1302−1306.

(38) Kosson, D. S.; Byrne, S. V. *Environ. Health Perspect., Suppl. 5* **1995**, *103*, 71−73.

(39) Balba, H. M.; Still, G. G.; Mansager, E. R. *J. Assoc. Off. Anal. Chem.* **1979**, *62*, 237−240.

(40) Worobey, B. L.; Webster, G. R. B. *J. Agric. Food Chem.* **1982**, *30*, 164−169.

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