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Document Title		
BIODEGRADATION OF TRIETHANOLAMINE IN NATURAL RIVER WATERS WITH COVER LETTER DATED 012893		
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Chemical Category		
TRIETHANOLAMINE (102-71-6)		



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Dear Sir or Madam:

As required by 40 CFR 716 as amended, we herewith submit a copy of a completed health and safety study.

Biodegradation of Triethanolamine in Natural River Waters.

<u>Chemical Name</u>	<u>CAS Number</u>
Ethanol, 2,2',2"-nitrilotris-	102-71-6

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Robert L. Hagerman
Research Associate
Regulatory Compliance
Health and Environmental Sciences
1803 Building
(517) 636-6855

86930000119

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PAGE 1 OF 30

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Study Title

BIODEGRADATION OF TRIETHANOLAMINE IN NATURAL RIVER WATERS

Author(s)

Personal Information
Personal Information

Study Completion Date

December 1, 1992

Performing Laboratory

Environmental Toxicology and Chemistry Research Laboratory
Health and Environmental Sciences
The Dow Chemical Company
Midland, Michigan 48674

Laboratory Study Number

Financial Information

Compound: TRIETHANOLAMINE

Title: BIODEGRADATION OF TRIETHANOLAMINE IN NATURAL RIVER
WATERS

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QUALITY ASSURANCE STATEMENT

Compound: TRIETHANOLAMINE

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WATERS

This study was examined for conformance with Good Laboratory Practices as published by the U.S. Environmental Protection Agency. The final report was determined to be an accurate reflection of the data obtained. The dates of Quality Assurance activities on this study are listed below.

Study Initiation Date: 05/15/92

Study Completion Date: 12/01/92

TYPE OF AUDIT: DATE OF AUDIT:

DATE FINDINGS REPORTED TO
STUDY DIRECTOR/MANAGEMENT:

Preliminary protocol 05/14/92

05/14/92

Final protocol 05/27/92

05/27/92

Study conduct 07/06/92

07/08/92

Protocol addendum 08/19/92

08/19/92

Protocol, data file
and draft report 11/24/92

11/24/92

ARCHIVING: Raw data and the final report are filed in the testing facility archives.

Personal Information (Date)
Quality Assurance
Health and Environmental Sciences
The Dow Chemical Company
1803T Building
Midland, Michigan 48674

SIGNATURE PAGE

Compound: TRIETHANOLAMINE

Title: BIODEGRADATION OF TRIETHANOLAMINE IN NATURAL RIVER
WATERS

Personal Information (Date)
Study Director

Personal Information (Date)

Reviewed by:

Personal Information (Date)

TABLE OF CONTENTS

	<u>PAGE</u>
SUMMARY.....	6
INTRODUCTION.....	7
MATERIALS AND METHODS.....	8
Test Chemicals.....	8
River Water/Sediment Collection.....	8
River Water/Sediment Biodegradation Experiments.....	9
River Water Biodegradation Experiments.....	9
Microcosm Analysis.....	9
Analytical Methods.....	10
River Sample Characterizations.....	11
Bacterial Enumeration Analysis.....	12
RESULTS.....	13
Sediment and Water Characterization.....	13
Biodegradation in Water/Sediment Mixtures.....	13
Biodegradation in River Water.....	15
DISCUSSION.....	16
REFERENCES.....	20
TABLES 1-2.....	22-23
FIGURES 1-7.....	24-30

SUMMARY

Biodegradation of [^{14}C]-triethanolamine (TEA) was observed in Tittabawassee and Chippewa River water and water/sediment mixtures at concentrations of 100 and 500 $\mu\text{g/L}$. Batch microcosm studies were conducted to assess the rate of biodegradation and degree of mineralization for TEA in these river systems.

Complete disappearance of 100 $\mu\text{g/L}$ TEA in water/sediment mixtures required less than two days in Tittabawassee River samples, and less than three days in Chippewa River samples. At the 500 $\mu\text{g/L}$ level, complete disappearance required less than five days in the Tittabawassee samples, and less than seven days in the Chippewa samples. The biodegradation of TEA in the water/sediment mixtures resulted in an initial accumulation of intermediate metabolite products, which degraded to near or below detection levels over less than nine days incubation. Recovery of $^{14}\text{CO}_2$ from the water/sediment mixtures was equivalent to between 49% and 63% of the original [^{14}C] activity.

Biodegradation of TEA in river waters alone was slower when compared to biodegradation in the water/sediment mixtures. Apparent lag periods of between six and 14 days were required before appreciable degradation occurred in the Tittabawassee River water. Complete disappearance of the TEA in river water samples required approximately 20 and 35 days incubation for the Tittabawassee and Chippewa River waters, respectively. TEA biodegradation in the river waters resulted in CO_2 as the only major metabolic product, as other intermediate products were present at near or below the detection levels.

Based on the results of these studies, TEA would be expected to rapidly biodegrade in a variety of natural rivers or streams.

INTRODUCTION

Triethanolamine (TEA) is widely used in consumer product formulations such as cosmetics, soaps, and household detergents. This compound is also used in industrial chelating agents, metal cutting fluids and polishing agents, and as a dispersant in agricultural formulations. A copper-TEA complex has been used as an aquatic herbicide. Because of its wide use and diverse applications, there exists a potential for TEA release to the environment.

The biodegradation of TEA has been examined in soil and municipal activated sludge (1). Rapid rates of biodegradation were observed in these environments, which is consistent with the large and highly diverse microbial populations present. Several other studies have demonstrated a slower biodegradation of TEA when exposed to environments containing lower numbers of microorganisms. Lamb and Jenkins reported only 6.8% of theoretical oxygen demand (ThOD) consumed after 20 days incubation in a standard biochemical oxygen demand (BOD) test using a dilute sewage inoculum and 2.5 ppm TEA (2). Brídíe (3) conducted a similar 5-day BOD test using acclimated and non-acclimated inoculum from a biological sanitary waste treatment plant effluent. Oxygen consumptions were 5% and 28% of ThOD for the non-acclimated and acclimated inocula, respectively. The biodegradation of TEA has also been examined in a variety of other tests which employ higher concentrations of microorganisms than the BOD test (4,5,6). Mills and Stack (4) conducted a 10 day die-away study using 50 ppm TEA and an acclimated Kanawha River water inoculated with sewage. They observed an oxygen consumption equal to 70% of theoretical oxygen demand (ThOD) after the 10 day period.

Until present, there has been little or no data available on the fate of TEA in natural waters. Data from BOD and similar tests suggest that biodegradation of TEA may be slower in environments that contain lower numbers of microorganisms, such as natural river waters. In the present study, the aerobic biodegradation of TEA in river water and river water/sediment mixtures was examined. The biodegradation of TEA was assessed using [¹⁴C]-

TEA, and the extent of mineralization was determined from the measurement of evolved $^{14}\text{CO}_2$ in sealed test microcosms. The identification of intermediate TEA degradation products was not attempted in this study.

MATERIALS AND METHODS

Test Chemicals

[^{14}C]-(U)- triethanolamine hydrochloride was obtained as an aqueous solution from New England Nuclear Research Products, Boston, MA (*Financial Information*). The radiochemical purity of this material was reported to be 99%, with a specific activity of 17.61 mCi/mmole.

All other chemicals and reagents used in this study were reagent grade and obtained from commercial sources. Water used in reagent and standard preparation was purified by a MilliQ water purification system (Millipore Corporation, Bedford, MA).

River Water/Sediment Collection

Authentic water and sediment samples used in biodegradation studies were collected from the Tittabawassee and Chippewa Rivers of central lower Michigan. The locations of these rivers and the collection sites are identified in Figure 1. Tittabawassee River samples were obtained on June 16, 1992 from a site near the Emerson Park boat ramp in Midland. Chippewa River samples were obtained on June 28, 1992 from a site approximately 300 feet downstream of the Meridian Road bridge, southwest of Mt. Pleasant. Water samples were collected immediately below the surface in sterile amber glass jugs. Sediment samples were collected from the top 1-2 inches of sediment immediately below the water collection sites. Upon collection, sediment samples were decanted of excess water and sealed in sterile containers. Tittabawassee River samples were transported to the laboratory immediately, and used within 24 hours of collection. The Chippewa River samples were stored on ice in a cooler immediately after collection and were used within 24 hours. River waters were used in degradation experiments as collected, without filtration. Sediment samples were cleaned of large debris and homogenized before use

in the degradation experiments. Moisture content of sediment used in biodegradation experiments was determined gravimetrically.

River Water/Sediment Biodegradation Experiments

The biodegradation of 100 and 500 µg/L TEA was examined in batch microcosms containing Chippewa and Tittabawassee River water/sediment mixtures. Test microcosms were prepared in sterile 100 mL glass serum bottles by combining 10 g (dry wt.) sediment, 49 mL river water, and 1 mL of an aqueous [¹⁴C]-TEA•HCl solution. The addition of aqueous [¹⁴C]-TEA•HCl solution to the microcosms resulted in either 1.3 x 10⁶ dpm/50 mL solution and a nominal 100 µg/L TEA, or 6.4 x 10⁶ dpm/50 mL solution and a nominal 500 µg/L TEA. Control microcosms were prepared in a similar manner, with formalin added at a final concentration of 2% wt. to inhibit biological activity.

River Water Biodegradation Experiments

The biodegradation of 100 and 500 µg/L TEA was examined in batch microcosms containing authentic Tittabawassee River water. The biodegradation of 100 µg/L TEA was also examined in similar microcosms containing Chippewa River water. Test microcosms were prepared in sterile 100 mL glass serum bottles by combining 49 mL river water and 1 mL of an aqueous [¹⁴C]-TEA•HCl solution. Control microcosms were prepared in a similar manner, with the exception that formalin was added at a final concentration of 2% wt. to inhibit biological activity.

All microcosms were sealed using chlorofluoropolymer-lined rubber septa and aluminum crimp-seal caps. Microcosms were incubated in a New Brunswick Scientific Model G-27 Psychrotherm incubator/shaker (New Brunswick Scientific, Edison, NJ) at 25 ± 1°C. Darkness was maintained in the incubator, and agitation was provided with rotational mixing at 100 r.p.m.

Microcosm Analysis

Duplicate test microcosms were periodically analyzed by high performance liquid chromatograph (HPLC) and liquid scintillation counting (LSC) to

determine the distribution of total [^{14}C] activity between TEA and degradation products. Single control microcosms were analyzed at the beginning, middle, and end of the experiments to determine the amount of TEA degradation due to abiotic processes. Prior to analysis, water/sediment microcosms were amended with 1 mL of a 1N NaOH solution through the septa and mixed for 15 minutes on a reciprocating shaker. These bottles were then centrifuged in a Speed Vac Concentrator (Savant Instruments, Inc., Farmingdale, NY) at approximately 3,000 rpm for 30 minutes to separate sediment from the aqueous phase. A portion of the aqueous phase was filtered through a 0.45 μm nylon membrane filter (Gelman Sciences, Ann Arbor, MI) and the filtrate was analyzed by HPLC and LSC. Water microcosms were manually shaken for 30 seconds prior to analysis, with contents filtered and analyzed in the same manner as above.

The evolution of $^{14}\text{CO}_2$ in the microcosms was periodically determined using a liquid scintillation analysis (7). This analysis was performed using duplicate microcosms and the apparatus schematically described in Figure 2. Nitrogen gas was purged through acidified microcosms at a rate of >250 mL/minute. Carbon dioxide in the exhaust gas was collected in a series of two traps containing 10 mL of a 1 N NaOH solution. River water and water/sediment microcosms were acidified through the septa by the addition of 1 mL and 2 mL 85% phosphoric acid, respectively. Microcosms were typically purged for at least 30 minutes to ensure a complete transfer of evolved CO_2 to the trap solutions. Trap solutions were combined and analyzed for total [^{14}C] content by LSC. The identity of $^{14}\text{CO}_2$ as the sole source of activity in the traps was confirmed by treating 5 mL of trap solution with 1 g barium nitrate and mixing for at least 30 minutes. The resulting mixture containing BaCO_3 precipitate was filtered through a 0.45 μm nylon membrane, and filtrate was analyzed by LSC to confirm the absence of [^{14}C] activity.

Analytical Methods

Filtered microcosm samples were analyzed for [^{14}C]-TEA and [^{14}C]-degradation products by HPLC. The HPLC analysis consisted of a strong cation exchange separation, which was achieved using a 4.6 mm \times 25 cm Partisil 10-

SCX column (Whatman International Ltd., Maidstone, Eng.). A mobile phase gradient was delivered through the column at 1.0 mL/minute using Waters 510 and 6000A pumps. Gradient solvent delivery was controlled by a Waters Model 680 solvent programmer (Millipore Corporation, Bedford, MA). The mobile phase contained a 0.05M KH_2PO_4 buffer, and the composition changed from 100% water to 100% (30/70 acetonitrile/water) over a 20 minute linear gradient. [^{14}C]-compounds in the column effluent were detected using a Berthold LB 506A on-line radioactivity monitor with 400 μL GT cell (Berthold Systems Inc., Pittsburgh, PA). Representative chromatograms for samples containing [^{14}C]-TEA and [^{14}C]-metabolites are shown in Figure 3.

Total [^{14}C] activity in CO_2 traps and filtered microcosm samples was determined using either a Beckman LS-9800 or LS-6000 liquid scintillation counter (Beckman Instruments, Inc., Fullerton, CA). Aliquots (200 μL) of the filtered microcosm samples were added to triplicate scintillation vials containing 10 mL Aquasol liquid scintillation cocktail (NEN DuPont, Boston, MA). Aliquots of the carbon dioxide traps (200 μL) were added to triplicate vials containing 10 mL Aquasol and 1 mL water (water added to improve solubility of 1N NaOH in Aquasol). The LSC analyses were standardized with respect to counting efficiency using samples prepared with a [^{14}C]-toluene standard (NEN DuPont, Boston, MA). These standard samples, and corresponding blank samples were prepared so as to have a composition similar to that of the actual samples being analyzed. Radioactivity (^{14}C dpm) was measured using a counting program which corrected for counting efficiency and quenching.

River Sample Characterizations

The total dissolved organic and inorganic carbon content of the Chippewa and Tittabawassee River water samples was determined using a Dohrmann DC-80 low temperature UV/persulfate carbon analyzer (Rosemount Analytical, Santa Clara, CA) (8). Samples were filtered through 0.45 μm nylon membrane filters prior to analysis. Total organic carbon was determined from the difference of measured total carbon and total inorganic carbon values.

The pH of river waters and water/sediment mixtures was measured using an Orion Model 601A Digital Ionalyzer and Orion Model 91-05 combination pH electrode (Orion Research Inc., Cambridge, Mass.). The pH of microcosm samples was measured using an Altex Model 3560 digital pH meter (Beckman Scientific Instruments, Irvine, CA) and Sargent-Welch Model S-30072-15-A combination pH electrode (Sargent-Welch Scientific Co., Skokie, IL).

Sediment samples from the Tittabawassee and Chippewa Rivers were sent to A&L Mid West Laboratories (Omaha, Nebraska) for physical and chemical analysis. The sediments were characterized with respect to texture, organic matter content (by combustion), cation exchange capacity, and inorganic nutrient content.

Bacterial Enumeration Analysis

The total bacterial populations of the Chippewa and Tittabawassee River waters were estimated using standard plate count determinations (9). Serial dilutions of non-filtered river water were prepared in sterile 1% Sodium pyrophosphate buffer (pH 7). Aliquots of these dilutions (100 μ L) were plated on triplicate Tryptone glucose extract agar plates. Total bacterial populations in the water/sediment mixtures were estimated using the same standard plate count method. Mixtures were prepared in sterile 100 mL serum bottles using 10 g (dry weight) sediment and 50 mL unfiltered river water. The bottles were vigorously mixed for several minutes on a reciprocating shaker to facilitate removal of surface-attached bacteria. A 1 mL aliquot of the resulting slurry was serially diluted in sterile 1% sodium pyrophosphate buffer (pH 7). These dilutions were plated on triplicate Tryptone glucose extract agar plates.

All plates were incubated at 25°C in darkness. Microbial colonies on the agar surfaces were periodically counted using a Fisher Accu-Lite Colony Counter (Fisher Scientific, Fair Lawn, NJ). After 14 days incubation, final counts were taken from plates having between approximately 30 and 300 total colonies.

RESULTS

Sediment and Water Characterization

The results of physical, chemical, and biological characterizations of the Tittabawassee and Chippewa River waters and sediments are given in Tables 1 and 2. Both sediments were classified as sand, with very similar mineral compositions. The organic matter content of the sediments was low, at 0.4 and 0.9% for the Chippewa and Tittabawassee, respectively. These values correspond to approximate organic carbon contents of 0.23% and 0.52%, respectively (10).

Standard plate counts of the river waters and water/sediment mixtures were conducted to provide a comparison of the total bacterial populations present in each matrix. Microbial populations of the Chippewa and Tittabawassee River waters were found to differ by approximately two fold. Tittabawassee River water was found to contain approximately 6.4×10^3 colony forming units (CFU)/mL, while the Chippewa River water was found to contain only 3.3×10^3 CFU/mL. A Tittabawassee River water/sediment mixture with a composition identical to that of the test microcosms was found to contain 4.2×10^6 CFU/mL slurry. A similar mixture of Chippewa River sediment and water contained 9.8×10^5 CFU/mL slurry. These results indicate that the Tittabawassee water/sediment microcosms contained nearly four times the total bacteria of the Chippewa water/sediment microcosms. Total dissolved organic carbon contents of the waters were nearly identical, at approximately 8 mg/L. The pH measurements of both rivers were approximately 7.7.

Biodegradation in Water/Sediment Mixtures

Figure 4 shows TEA degradation in the Tittabawassee River water/sediment mixtures at 100 and 500 $\mu\text{g/L}$ levels. At the 100 $\mu\text{g/L}$ level, 96% of the original TEA disappeared after only one day. A slower rate of degradation was observed at the 500 $\mu\text{g/L}$ level, with 80% removal occurring over the same one day period. Recovery of $^{14}\text{CO}_2$ from the Tittabawassee River

water/sediment mixtures was equivalent to 49% of the original [^{14}C] activity after seven days for 100 $\mu\text{g/L}$ TEA, and 58% after nine days for 500 $\mu\text{g/L}$ TEA.

Figure 5 shows TEA degradation in Chippewa River water/sediment mixtures at 100 and 500 $\mu\text{g/L}$ TEA. Biodegradation was somewhat slower in these mixtures, as compared to those of the Tittabawassee. This is consistent with the lower total microbial populations present in the Chippewa River mixtures. After two days, 94% of the TEA was removed at the 100 $\mu\text{g/L}$ level. TEA removal of 57% was observed over the same two day period at the 500 $\mu\text{g/L}$ level. Recovery of $^{14}\text{CO}_2$ from the Chippewa River mixtures was equivalent to 52 and 63% of the original [^{14}C] activity after 15 days for initial TEA levels of 100 and 500 $\mu\text{g/L}$, respectively. Disappearance of TEA in the test microcosms was attributed to biological processes, as the concentration of TEA in biologically inhibited controls did not fall below 91% of the original concentration over the duration of the experiments.

"Products" as shown in the graphs of Figures 4, 5, and 6 represents the total [^{14}C]-activity from all intermediate and mineralization products (CO_2) detected in the HPLC analyses. The HPLC analysis detected only those compounds which were dissolved in the microcosm aqueous phase, and did not account for gaseous products in the microcosm headspace. Thus, relatively high values for "products" given at the end of each experiment actually reflect the high levels of dissolved [^{14}C]-carbonate species and low levels of [^{14}C]-intermediate organic products present in the microcosms. " CO_2 " as shown in the figures represents the total ^{14}C -activity due to mineralized organic [^{14}C] species in the aqueous phase and headspace of the microcosms.

Degradation rates in water/sediment mixtures were calculated from the initial rates of TEA disappearance and expressed as $\mu\text{g TEA L}^{-1} \text{Day}^{-1}$. The degradation rates in Tittabawassee River water/sediment mixtures were approximately 96.5 and 243 $\mu\text{g TEA L}^{-1} \text{Day}^{-1}$ for initial TEA concentrations of 100 and 500 $\mu\text{g/L}$, respectively. The corresponding degradation rates in Chippewa River water/sediment mixtures were approximately 46.8 and 140

$\mu\text{g TEA L}^{-1} \text{ Day}^{-1}$. The degradation of TEA in river water/sediment mixtures appeared to be dependent upon initial TEA concentration and total initial bacterial population present. Rates calculated for the Tittabawassee River mixtures were approximately a factor of two greater than those observed in corresponding Chippewa River mixtures. Correspondingly, the total bacterial population was approximately four times greater in the Tittabawassee River mixtures than in the Chippewa River mixtures.

Biodegradation in River Water

Biodegradation of TEA was observed in Tittabawassee River water at the 100 and 500 $\mu\text{g/L}$ levels. Figure 6 gives a graphic representation of the TEA biodegradation observed in Tittabawassee River water microcosms. At the 100 $\mu\text{g/L}$ level, TEA biodegradation was slow, with an apparent lag period of approximately 10 to 14 days. Following the lag period, greater than 80% disappearance of TEA was observed over the next 10 days. TEA degradation at the 500 $\mu\text{g/L}$ level was also slow, with an initial lag period of approximately six to 10 days. This was followed by rapid degradation of TEA, with greater than 95% disappearance of the compound over the next 14 days.

The biodegradation of 100 $\mu\text{g/L}$ TEA in Chippewa River water is shown in Figure 5. The disappearance of TEA in Chippewa River water was slower than observed in Tittabawassee River water. A 96% removal of 100 $\mu\text{g/L}$ TEA in Chippewa water required 31 days, versus less than 20 days in the Tittabawassee water. This is consistent with the lower number of microorganisms present in the Chippewa River water. A lag period was not apparent in studies using the Chippewa River water.

TEA was mineralized to CO_2 in the Tittabawassee and Chippewa River water microcosms. Recovery of $^{14}\text{CO}_2$ in the 100 and 500 $\mu\text{g/L}$ microcosms after 21 days averaged 64% and 58% of the initial ^{14}C activity, respectively. The degradation of TEA resulted in minimal formation of intermediate degradation products. HPLC analyses of the river water microcosms consistently showed peaks where ^{14}C -TEA and ^{14}C -carbonate species eluted.

[¹⁴C]-intermediate products other than ¹⁴CO₂ species were typically present, but at near or below their detection limits. The disappearance of TEA in the river water microcosms was attributed to biological processes, as control microcosms showed only slight losses of TEA over the duration of the experiments.

Degradation rates in river water were calculated using the rate of disappearance observed after the apparent lag periods. The rates were expressed as $\mu\text{g TEA L}^{-1} \text{Day}^{-1}$. The degradation rates in Tittabawassee River water were approximately 7.8 and 32 $\mu\text{g L}^{-1} \text{Day}^{-1}$ for initial TEA concentrations of 100 and 500 $\mu\text{g/L}$, respectively. The degradation rate in Chippewa River water was calculated to be approximately 3.8 $\mu\text{g L}^{-1} \text{Day}^{-1}$ for an initial TEA concentration of 100 $\mu\text{g/L}$. The degradation of TEA in river water appeared to be dependent upon both initial TEA concentration and total number of microorganisms present. The Tittabawassee River water was found to contain nearly twice the total bacteria as the Chippewa River water. The rate of disappearance of 100 $\mu\text{g/L}$ TEA in Tittabawassee River water was approximately twice that observed for the same concentration in Chippewa River water.

DISCUSSION

This study has shown TEA to be biodegraded in natural river water/sediment mixtures at initial concentrations of 100 and 500 $\mu\text{g/L}$. The rate of TEA degradation was lower in microcosms containing only river water. In the water/sediment matrices, the disappearance of TEA resulted in an initial accumulation of intermediate degradation products. However, the levels of these intermediate products declined with time and a corresponding increase in ¹⁴CO₂ was observed. In the river water, CO₂ was the major degradation product, and intermediate degradation products were present at near or below their detection levels. The degradation of TEA and its metabolites was biologically mediated, as negligible loss of TEA was observed in killed control systems. After complete disappearance of TEA, yields of ¹⁴CO₂ from the microcosms continued to increase with further disappearance of the intermediate products.

The oxidation of a variety of substituted and non-substituted alkyl amines by microorganisms such as yeast and bacteria has been reported previously (11,12,13). In one study, Williams and Callely isolated a Gram-negative rod-shaped bacterium from activated sludge which was capable of utilizing ethanolamine, diethanolamine, and triethanolamine as sole sources of carbon and energy (14). They found that enzymes responsible for the oxidation of these compounds were induced in the bacterium after short acclimation periods. Whole-cell extracts of these acclimated cells were compared to identical cells which were not growing on the ethanolamines. Enzymes thought to be responsible for primary metabolism of these compounds were found at high levels in exposed cells, while the same enzymes were not present in non-exposed cells. The proposed pathway for metabolism of the ethanolamines by this bacterium is shown in Figure 7 (14). This pathway is nearly identical to one proposed by Fattakhova et al. for the catabolism of the same compounds by the yeast *Rhodotorula mucilaginosa* (15). Although the present study did not attempt to identify TEA metabolism products, the chromatographic behavior of the products observed in this study is consistent with what may be expected from the products described in Figure 7.

Variable results have been reported where biodegradation of TEA was examined in BOD and similar tests. Results of these tests suggest that the inoculum source and extent of acclimation are critical factors influencing the biodegradation rates. For example, a BOD test performed by Lamb and Jenkins using a dilute non-acclimated sewage inoculum resulted in only 6.8% of ThOD consumed after 20 days (2). On the contrary, Bridie (3) observed oxygen consumptions as high as 28% of ThOD after only five days, using a dilute acclimated sewage inoculum. The occurrence of apparent lag periods in the present river water studies supports these previous studies that suggest acclimation plays a key role in governing TEA biodegradation. The apparent lag periods observed in the river water studies may be due to the time required for induction of enzymatic systems capable of degrading TEA. Similar observations for the degradation of alkanolamines were reported by Williams and Callely (14). The lag periods may also be attributed to time

required for the low levels of bacteria to increase in number for a measurable removal of TEA to be observed.

The concentrations of TEA examined in the biodegradation experiments were chosen to represent concentrations that may occur in a natural fresh water river or stream. Dissolved TEA in a river or stream would be expected to be in contact with both planktonic and sediment-associated microorganisms. Based on its high water solubility, TEA would be expected to remain dissolved in the water column and would not undergo appreciable adsorption or partitioning into sediments. For these reasons, the degradation rates calculated for the water/sediment system are thought to more closely represent what would be observed in an actual river or stream.

An actual river or stream system is difficult to simulate in the laboratory. However, a microcosm study of rivers with different physical and biological characteristics gives insight into the chemical and biological processes that would actually occur in these environments. The sampling sites on the Chippewa and Tittabawassee Rivers were specifically chosen to represent two characteristically different river systems. Although both rivers flow through the same general geographic area, physical characteristics such as depth, width, flow rate, and sources of drainage input differ greatly. Microbial populations of the two river waters were similar; however, the microbial population associated with the sediments were found to differ greatly. The Chippewa River site is characteristic of a typical Michigan trout stream, with clear, swift flowing, shallow water and a gravelly river bottom. The Chippewa drains mostly abandoned farmland and wooded areas, which limit inputs of sediment-laden runoff. Factors such as fast current and limited biological input from farmland drainage/runoff would tend to keep microbial populations in the sediment and water low. In contrast, the Tittabawassee River site is characteristic of this deep, wide, slow-flowing river that drains a large portion of Midland County's active farmlands. The river is characterized by a sand/silt bottom and fairly opaque, sediment-laden waters. Factors such as slow flow, sand/silt sediment, and relatively high inputs from agricultural drainage/runoff would tend to promote higher numbers of

microorganisms associated with the water and sediment. The results of these studies for two physically and biologically different river systems provides insight into the effect which these types of factors might have on the fate of TEA. Based on these results, TEA would be expected to rapidly biodegrade in a variety of natural rivers or streams.

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Table 1: Chemical and Biological Characteristics of River Waters

<u>Parameters</u>	<u>Tittabawassee River</u> <u>Water</u>	<u>Chippewa River</u> <u>Water</u>
pH	7.7	7.7
Total Dissolved Organic Carbon	8.1 ppm	8.7 ppm
Total Dissolved Inorganic Carbon	48.7 ppm	46.6 ppm
CFU/mL	6.4×10^3	3.3×10^3

Table 2: Physical, Chemical, and Biological Characteristics of River Sediment*

<u>Parameters</u>	<u>Tittabawassee River Sediment</u>	<u>Chippewa River Sediment</u>
Texture (%)		
Sand	90	96
Silt	4	0
Clay	6	4
Organic Matter % by Combustion	0.9	0.4
pH	8.1	7.8
Nutrients (ppm)		
NO ₃ -N	2	2
P (Weak Bray)	15	6
P (Strong Bray)	24	10
S	24	10
Zn	0.9	1.2
Cu	0.5	2.7
B	1.0	0.7
CFU/mL Mixture	4.2 x 10 ⁶	9.8 x 10 ⁵

*Physical and chemical analyses were performed by A&L Mid West Laboratories, Omaha, NE.

Figure 2: Schematic of Apparatus Used in $^{14}\text{CO}_2$ Microcosm Analysis

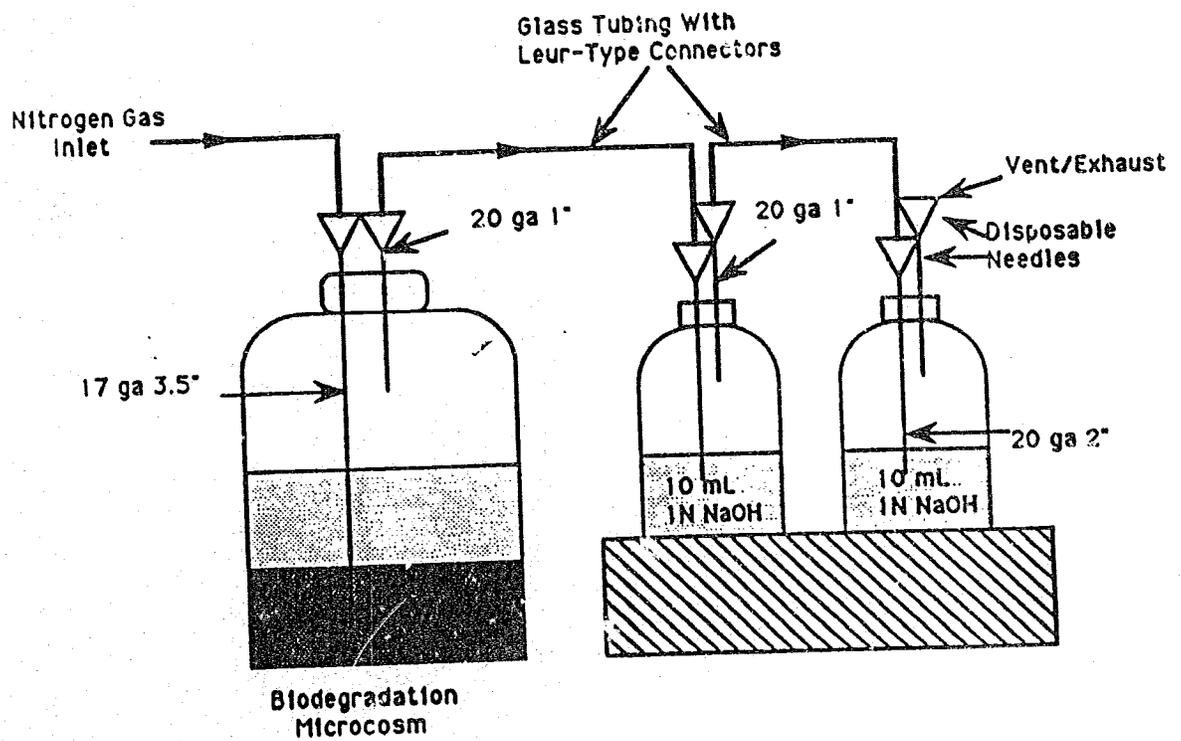


Figure 3: Representative HPLC Analyses of [¹⁴C]-TEA and [¹⁴C]-Degradation Products

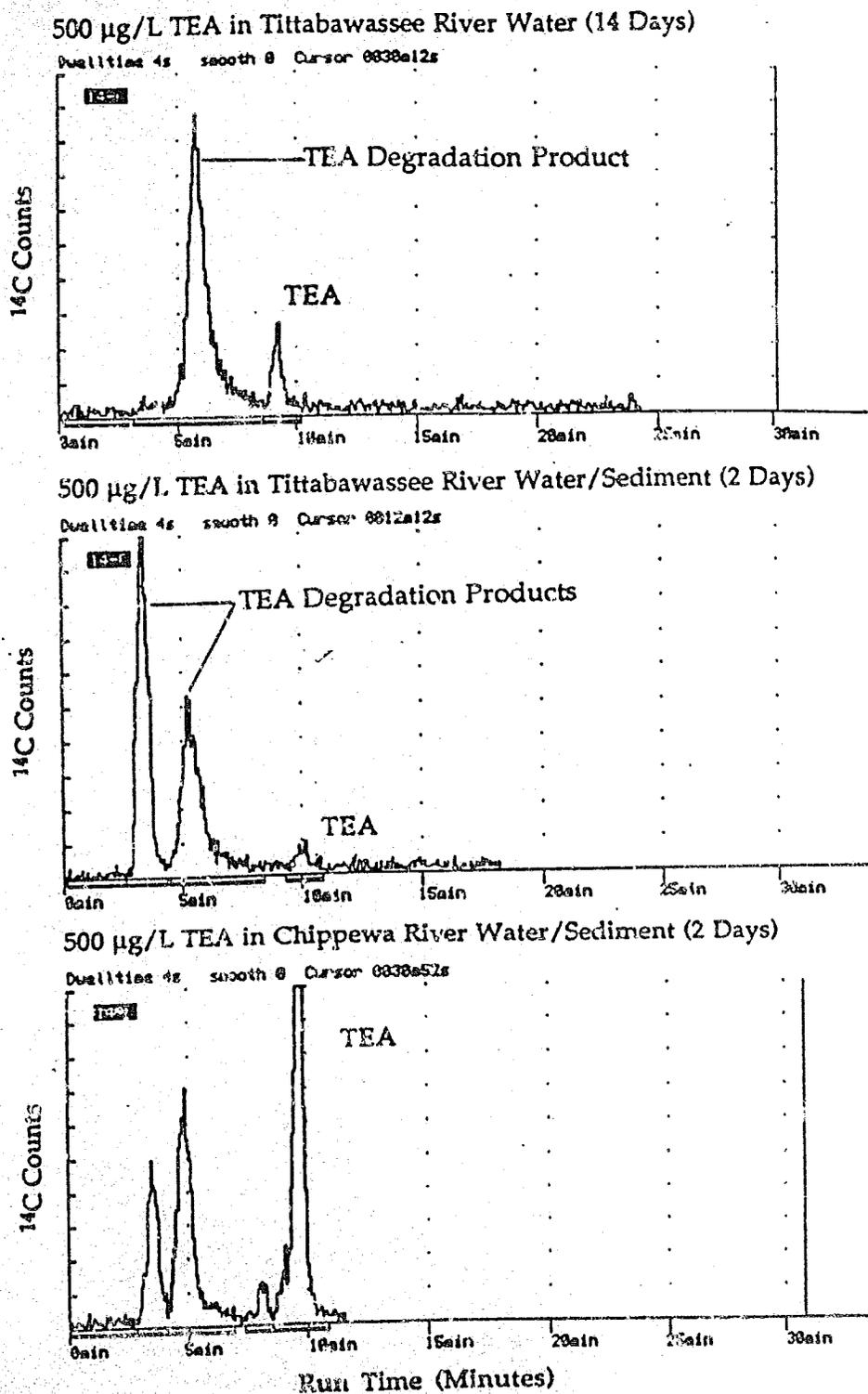


Figure 4: Biodegradation of 100 (A) and 500 $\mu\text{g/L}$ (B) TEA in Tittabawassee Water/Sediment Mixtures

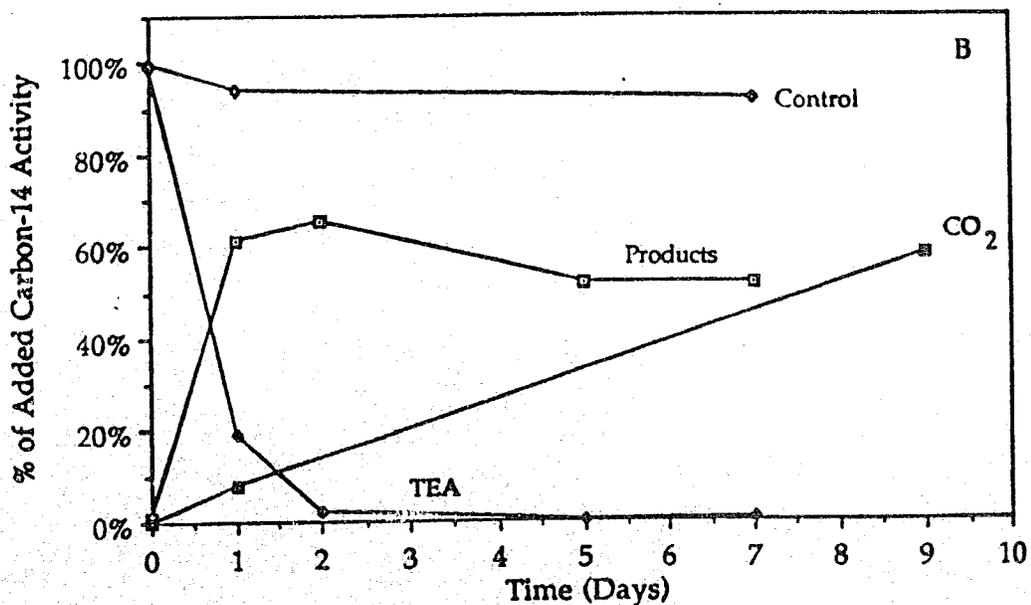
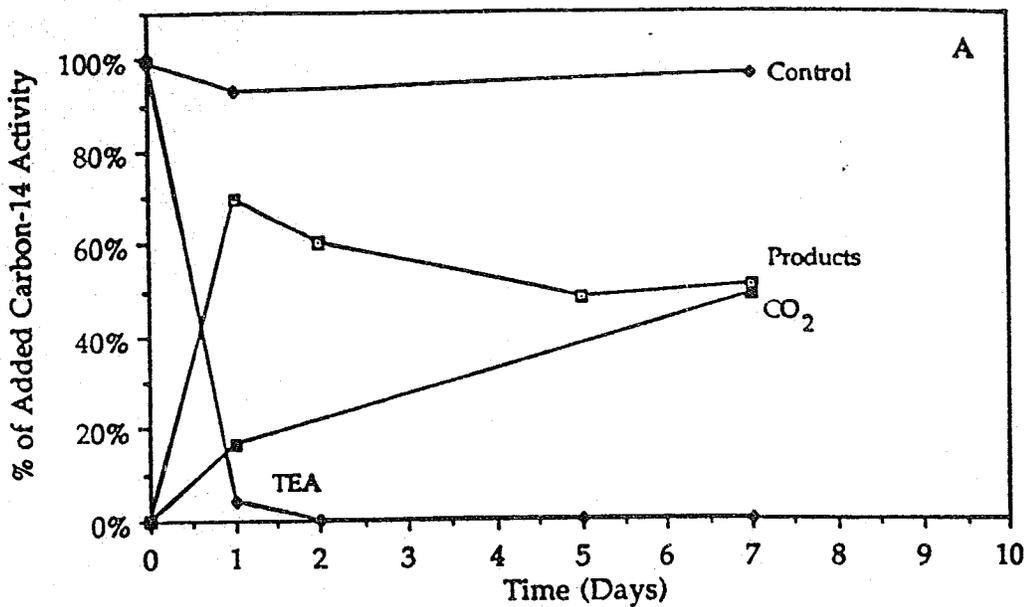


Figure 5: Biodegradation of TEA in Chippewa River-(A) 100 $\mu\text{g/L}$ in Water/Sediment, (B) 500 $\mu\text{g/L}$ in Water/Sediment, (C) 100 $\mu\text{g/L}$ in Water

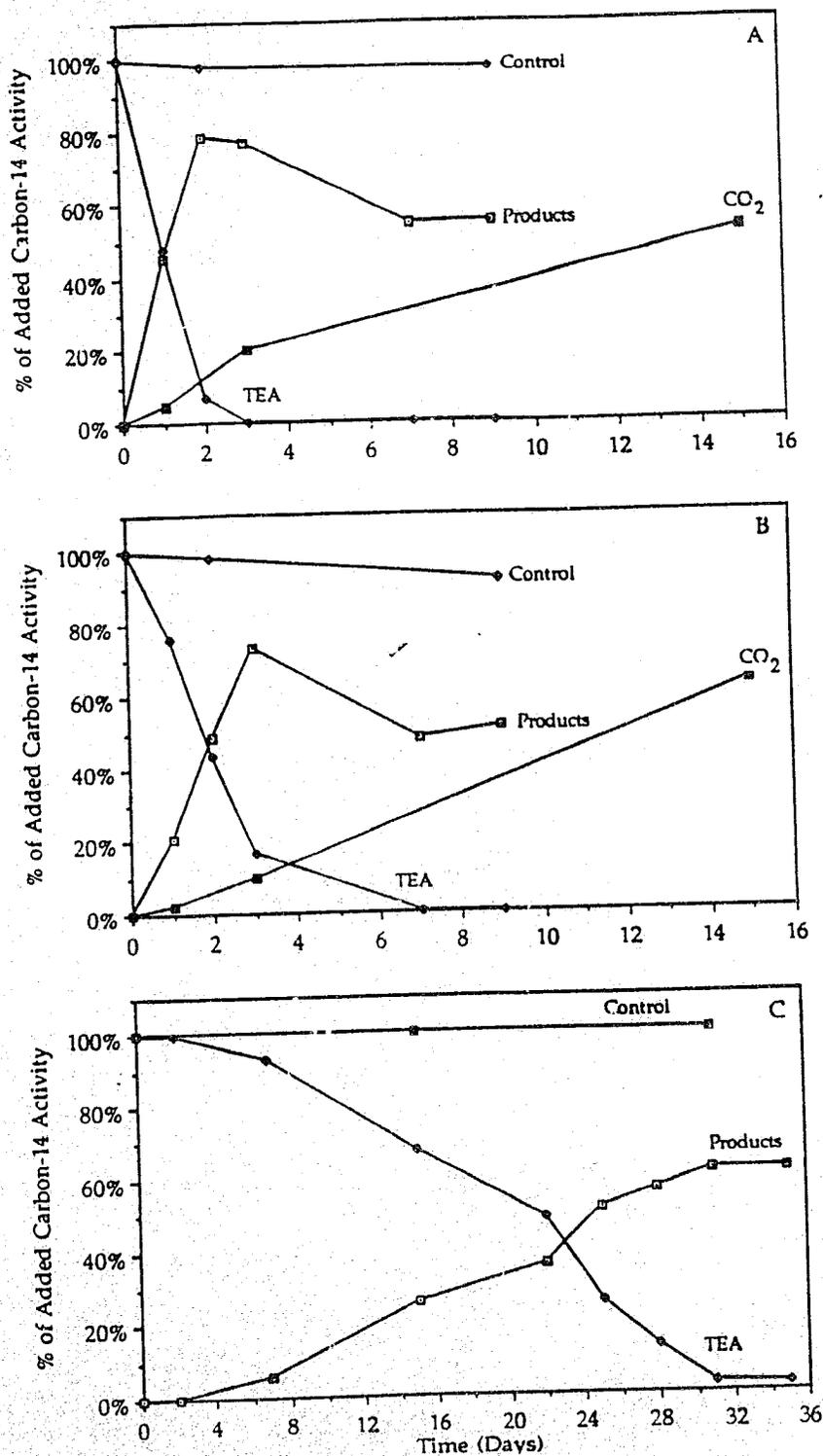


Figure 6: Biodegradation of 100 (A) and 500 $\mu\text{g/L}$ (B) TEA in Tittabawassee River Water

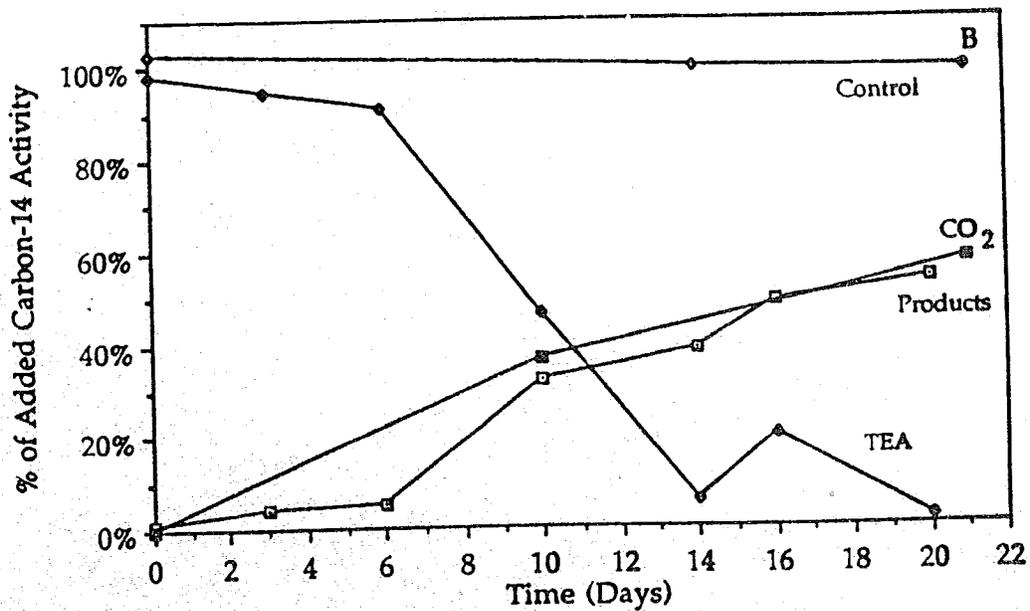
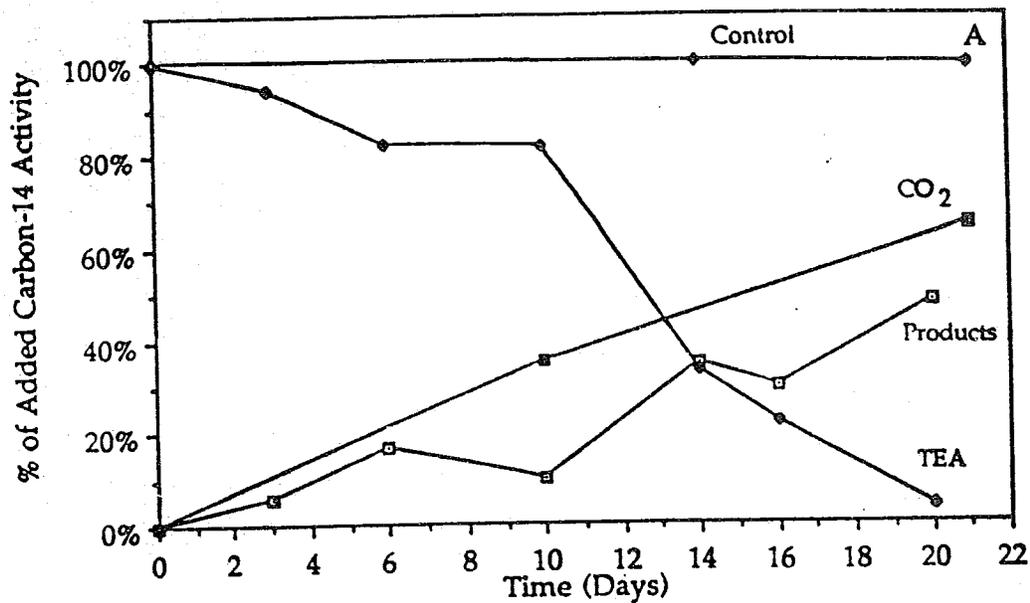
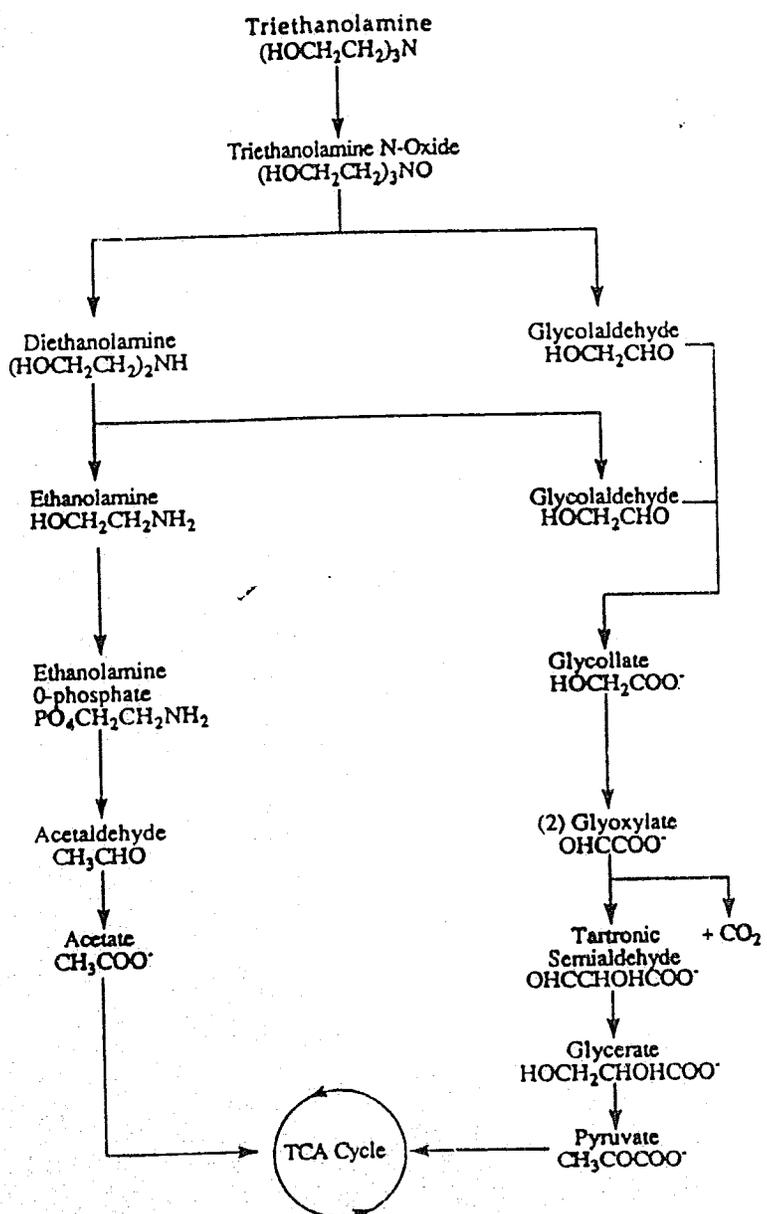


Figure 7: Proposed Pathway for the Metabolism of Triethanolamine, Diethanolamine, and Ethanolamine by a Gram-negative Rod (14)



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