MRI ❈ REPORT

IDENTIFICATION OF ANALYTICAL PROCEDURES FOR NITRILOTRIACETIC ACID (NTA)
IN WATER, SEDIMENT, AND DRINKING WATER

TASK 40
FINAL REPORT
November 2, 1981

EPA Prime Contract No. 68-01-5915
MRI Project No. 4901-A(40)

Prepared for:

U.S. Environmental Protection Agency
Office of Pesticides and Toxic Substances
Field Studies Branch TS-798
401 M Street, S.W.
Washington, D.C. 20460

Attn: Dr. Frederick Kutz, Project Officer
Mr. Daniel Heggem

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by

Viorica Lopez-Avila
John E. Going

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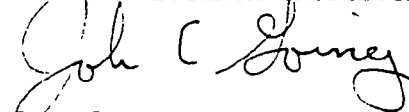Mr. Daniel Heggem

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PREFACE

This report presents the results of the literature review accomplished on MRI Project No. 4901-A, Task 40, "Identification of Analytical Procedures for Nitrilotriacetic Acid (NTA) in Water, Sediment, and Drinking Water," for the Environmental Protection Agency (EPA Prime Contract No. 68-01-5915). The review was performed by Dr. Viorica Lopez-Avila. The report was prepared by Dr. Lopez-Avila with assistance from Dr. John Going. Dr. Mitchell Erickson contributed to the final editing.

Midwest Research Institute would like to thank Drs. Larry Games and Gil Cloyd from Procter and Gamble for providing valuable assistance during the course of this task. The cooperation of personnel from Monsanto Company (Mr. Ed Malec) and W. R. Grace Company (Mr. J. Amairsakris) is sincerely appreciated.

MIDWEST RESEARCH INSTITUTE

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SECTION 1

SUMMARY

The purpose of this task was to conduct a comprehensive literature review of analytical techniques for nitrilotriacetic acid (NTA) in water, sediment, and drinking water and to summarize the readily available monitoring data. This literature review identified promising analytical techniques for NTA and at the same time pointed out deficiencies and voids in the current methods.

Fifty articles dealing with analysis of NTA in various matrices (e.g., water, drinking water, sewage sludge, etc.) were retrieved from the open literature following a computerized literature search and contacts with private companies. Areas which were specifically addressed in obtaining the pertinent information were: compound stability, isolation from the matrix (extraction efficiency, removal of matrix interferences), derivatization procedures for gas chromatographic analysis, selectivity and sensitivity of the detection system, and examples of monitoring studies.

The most promising analytical procedure, still under evalution by Procter and Gamble Company, involves concentration of NTA from water samples using anionic ion exchange resins, elution with formic acid, derivatization to tri-n-butyl NTA, and analysis by gas chromatography with nitrogen selective detector (GC/NPD). Confirmation is done by gas chromatography/mass spectrometry (GC/MS). Use of ^{14}C-labeled NTA spiked into water samples directly in the field or prior to the isolation step allows correction of NTA recovery from the sample matrix by scintillation counting measurement. Quantitation of NTA by GC/NPD and GC/MS in the selected ion monitoring mode was comparable, although daily fluctuations of the NPD response were reported. Use of an internal standard such as nitrilotripropionic acid (NPT) resulted in improved consistency of the response factors. This method gave improved precision and accuracy in influent sewage, effluent sewage, river water, and tap water at concentrations < 100 µg/liter. Other analytical procedures discussed in the report involve polarography, colorimetry, and potentiometry. Difficulties in the determination of NTA by these techniques which arise from interference by metals and naturally occurring ligands, such as ethylenediamine tetraacetic acid (EDTA), are also discussed.

No analytical procedures to analyze for NTA in river sediments were reported in the literature. Extraction of NTA from sediment with water, dilute aqueous base, or organic solvent may be applicable. Once extracted in an aqueous solution or an organic solvent, analytical procedures described for water can be applied.

Monitoring data for NTA in the Canadian environment and data regarding removal of NTA by activated sludge treatment are presented. The results of the Canadian monitoring program indicate relatively few locations where NTA levels occurred above 10 µg/liter. The authors of the study concluded that use of NTA as detergent builder is not likely to increase the levels of NTA in the Canadian environment.

SECTION 2

RECOMMENDATIONS

• The analytical procedure involving concentration of NTA from water by
ion exchange chromtography, elution with formic acid, derivatization to a
volatile ester, and analysis by gas chromatography is recommended to analyze
for NTA at microgram-per-liter concentrations in water samples.

• Additional method development should address sample preservation pro-
cedures and NTA isolation from waters and sediments. Preservation of samples
by addition of formaldehyde solution was frequently reported; however, no jus-
tification for selection of this preservative was provided. Other preserva-
tion reagents, such as formic acid, should be investigated. Investigation of
other ion exchange resins that would allow concentration of larger volumes of
water samples and thus lower detection limits for NTA should also be consid-
ered. The necessity and methods for treatment of glassware to prevent NTA
losses should be further studied.

• Use of ^{13}C-labeled NTA to be spiked in the field is strongly recom-
mended; this is expected to improve the method precision and accuracy since
the recovery of NTA can be corrected by the recovery of ^{13}C-labeled NTA added
to the sample, as measured by GC/MS.

• The GC/MS technique is preferred to GC/NPD technique especially if ^{13}C-
labeled NTA is to be added to the sample. This will allow both positive con-
firmation and quantitation of NTA.

• Use of capillary columns to chromatograph the water or sediment ex-
tracts containing the NTA ester should be investigated. The higher resolving
power of a capillary column over the packed column may help to reduce the need
for sample cleanup and increase the method sensitivity.

SECTION 3

INTRODUCTION

Interest in the determination of NTA first occurred as a consequence of the observation that small amounts of NTA had adverse effects on the quality of indicator color change in some complexometric titrations.[1] After its introduction as a partial replacement for sodium tripolyphosphate in detergent formulations, the interest in the NTA determination shifted to drinking water, inland waters, sewage, etc., because of the increased concern over effects of NTA in the environment.

In the United States NTA was marketed as a detergent builder in the 1960's. However, in December 1970, the use of NTA in detergent formulations was discontinued in the United States as the result of reported adverse health effects. Despite this fact, Canada and Sweden continued to use NTA after a thorough evaluation of all available toxicological data.

Currently NTA is manufactured by Monsanto and W. R. Grace who export it to Canada and supply it for use in certain industrial processes.[2] When restrictions were placed on the use of phosphates in Canada, the use of NTA increased. For example, early in 1972 the NTA content in detergents was 6%; whereas in 1973 the average content was 15%. Consequently, the discharge of NTA to the environment increased. It was estimated that a city with a population of one million would discharge 5,000 lb of NTA per day with an average concentration in the wastewater of 5 ppm.[3]

In order to determine whether NTA discharged with the wastewaters would build up in the aquatic system, a National Surveillance Program was initiated in Canada to monitor levels of NTA in raw and drinking waters.[3] The results of this study demonstrated that NTA did not appear to accumulate in the Canadian water sources. During a 5-year period, the accumulated data indicate relatively few locations where NTA levels occur above 10 µg/liter.

In the United States, at industry's request, the Office of Testing and Evaluation (OTE) reviewed risks associated with NTA. Based on findings of this risk assessment, the Environmental Protection Agency (EPA) has decided not to take any regulatory action against the resumed use of this compound in laundry detergents. They recommend that NTA manufacturers and processors conduct monitoring and environmental studies in order to confirm predictions that NTA does not accumulate in the environment.

The purpose of this task was to conduct a comprehensive literature review of analytical techniques for NTA in water, sediment, and drinking water.

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This literature review was intended to identify techniques available for the analysis of NTA in water and sediment and, at the same time, to point out the deficiencies and voids in the current methods. The information regarding NTA analysis was retrieved from the open scientific literature, government reports, and unpublished literature from private companies contacted directly. The next section summarizes the analytical methods, and the final section presents NTA monitoring data.

SECTION 4

ANALYTICAL TECHNIQUES FOR NTA IN WATER, SEDIMENTS AND DRINKING WATER

A summary of published methods for NTA analysis is given in Table 1. Examination of this table shows a wide range of detection methods (e.g., gas chromatography, high pressure liquid chromatography, polarography, colorimetry, potentiometric titration, etc.) that vary in sensitivity, selectivity, complexity, degree of evaluation, ease of operation, etc. A detailed discussion of each of these techniques follows. Sample preservation and NTA isolation from water and sediment are discussed first.

SAMPLE PRESERVATION

The importance of proper sample preservation cannot be overemphasized. NTA was reported to be easily biodegradable and loss during sample shipment and storage is expected if samples are not preserved or maintained at low temperatures (< 5°C). The reported values of the half-life of NTA in receiving waters vary from minutes to months,[3] being affected by the presence of heavy metals, and the biological activity of the matrix.

This section describes procedures reported in the literature for preserving water samples containing µg/liter levels of NTA. These include: addition of formaldehyde, sterilization by filtration and freezing, and ultraviolet light treatment.

The most common preservation technique uses formaldehyde (1% solution of 37% solution) and storage at 5°C prior to analysis.[8,9] Storage at room temperature in polyethylene bottles following preservation with 1% formaldehyde solution was also reported.[12] In spite of the extensive study surveying NTA in the Canadian drinking waters,[3,9] no details about the stability with time of low levels of NTA in distilled water and natural water, preserved with formaldehyde solution, were given. An unpublished memorandum from L. M. Games at Procter and Gamble, Cincinnati, Ohio, reports that formaldehyde is stabilized for "long periods of time" by addition of 1% formalin to samples stored in polyethylene.

TABLE 1. SUMMARY OF PUBLISHED METHODS FOR NTA ANALYSIS

Description	Reference

Gas chromatographic techniques

*   Specific method for NTA, ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA). Aqueous sample is evaporated to dryness. NTA is derivatized with CH_3OH/BF_3. Analysis by GC/FID. Concentrations lower than 200 µg/liter NTA could not be detected.   4

*   Gas chromatographic determination of NTA as trimethyl ester. Inland water samples. Lower limit of detection 25 µg/liter. Identity of ester confirmed by GC/MS.   5

*   Gas chromatographic determination of NTA as tripropyl ester. Aqueous sample is evaporated to dryness and treated with an n-propanol/acetyl chloride (10:1) mixture. The resulting NTA-propyl ester is analyzed using a nitrogen phosphorus detector. Concentrations down to 1 µg/liter NTA were detected in tap and river water.   6

*   Gas chromatography of tri-propyl ester with prior concentration on ion exchange resin. Microgram level. Determination in lake waters. No interference from fatty acids.   7

*   NTA was determined in aqueous systems ranging from tap water to sewage effluents by use of anion-exchange resins, derivatization with n-butanol/HCl and gas chromatography. Concentrations at 1 to 10,000 µg/liter were reported.   8

*   Determination of NTA using gas chromatography with nitrogen specific detector was evaluated and applied to estimate the levels of NTA in Canadian raw and potable waters. Concentration of NTA from water using ion exchange resins and derivatization to its tri-n-butyl ester were used (Aue's Method, Ref. 8). Limit of detection 0.2 µg/liter.   9

*   NTA was analyzed at sub-µg/liter levels in raw water and drinking water using gas chromatography with nitrogen-selective detector. Aue's Method (Ref. 8) was used to concentrate NTA from the aqueous sample.   10

*   Gas chromatographic determination as tri-n-butyl ester after anion-exchange chromatography. Quantitative analysis at 10 µg/liter demonstrated NTA was separated as its tri-n-butyl ester from 24 other fatty acid, phenolic, and poly-carboxylic acids using OV-210 columns.   11

(continued)

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TABLE 1. (continued)

Description	Reference

* Gas chromatographic determination with nitrogen selective detection of NTA as its tri-n-butyl ester after anion exchange chromatography. Method gives improved precision and accuracy in influent sewage, effluent sewage, river water, tap water at concentrations < 100 µg/liter. Variations in recovery due to matrix effects are overcome by the use of ^{14}C-NTA as a tracer. Identification of NTA confirmed by GC/MS in the selected ion monitoring (SIM) mode.   12

* Gas chromatographic determination of NTA and related amino-polycarboxylic acids as n-butyl and n-trifluoroacetyl, n-butylester derivatives. Limit of detection 10 µg/liter for all compounds. Identification by GC/MS. Applied to sewage influent, inland waters.   13

* Method based on the gas chromatographic determination of NTA as its trimethylsilyl derivative. The sample is evaporated to dryness directly in the reaction vial with an excess of the ammonium salt of EDTA to mask interfering cations and derivatized with bis(trimethylsilyl)trifluoro-acetamide in dimethylformamide. Method is suitable to samples containing 1 to 100 mg/liter NTA without a prior concentration step.   14, 15

* Gas chromatographic determination of NTA esterified with N,O-bis(trimethylsilyl)acetamide. Confirmation by GC/MS.   16

* Gas chromatographic determination in natural waters as tris(2-chloroethyl ester). Specificity claimed in sewage samples. Preconcentration on anion-exchange resin; elution with HCl. Identity of ester confirmed by GC/MS.   17

High pressure liquid chromatography

* Anion exchange chromatography was investigated to separate NTA from other amino-acid chelates. Possible interferences from metallic ions were overcome by converting all metal-NTA chelates to ferric chelate. Method applied to analysis of sewage samples and solutions of detergent formulations with a sensitivity of 1 mg/liter.   18

Polarographic techniques

* Polarography with cadmium. NTA is converted to its 1:1 cadmium complex by adding a 100% excess of cadmium. Determination of NTA in lake waters (1 to 10 mg/liter).   19

(continued)

TABLE 1. (continued)

Description	Reference
* Differential cathode-ray polarography was used to determine "free" NTA in natural water as the lead-NTA complex. Determination of "total" NTA is based on releasing NTA from metal complexes under acidic conditions. Subsequent addition of EDTA and adjustment of pH to 8 preferentially bind the metals with EDTA. Detection limits of 10 μg/liter were reported without any sample preconcentration.	20
* Automated method for the determination of NTA in natural waters, detergents, and sewage samples, based on the formation of bismuth NTA complex at pH 2 using twin cell oscillographic DC polarography; 15 samples/hour can be processed. Method can be used to detect 10 μg/liter without any sample preconcentration.	21
* Polarographic analysis of NTA as In(III)-NTA complex. The method was evaluated with river water and sewage samples at 25.7 ppb to 2.57 ppm using anion exchange concentration and isotope dilution to correct for incomplete recovery. No interference observed with a 10-fold molar excess of EDTA and EHDP (ethane-1-hydroxy-1,1-diphosphonate), citrate, maleate, phosphate, and carbonate. No apparent interference from surfactants.	22
* Polarographic determination of NTA as Pb-NTA complex, Cd-NTA complex, and Bi-NTA complex was evaluated. Pb-NTA complex was investigated in three buffer systems: tris-hydro-chloride-ammonium hydroxide, glycine-ammonium hydroxide, and ammonium chloride-ammonium hydroxide, at pH 7 to 8. Cd-NTA complex was measured in ammonium chloride-ammonium hydroxide buffer at pH 9. Bi-NTA complex was investigated in NaCl media at pH 2. Polarographic determination of NTA as the Bi-NTA complex was found to be the most sensitive (50 μg/liter detection limit).	16
* NTA was determined in natural and wastewaters at 3 μg/liter as the bismuth complex using dual cell pulse differential polarography. Effects of possible interference by metal ions and complexants were evaluated.	23
* Differential pulse polarography of NTA and EDTA as Cd-complex in synthetic seawater and phytoplankton media. The presence of competing metal cations, including copper is not detrimental if the method of standard additions is used.	24

(continued)

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TABLE 1. (concluded)

Description	Reference

Colorimetric techniques

* Determination in soils. Soil sample extracted with 0.025 M NaOH and CaCl$_2$ solution. Extract analyzed for NTA by photometric measurement of blue color (borate buffer, pH 9.2) developed by modifying the zinc-Zincon method. Calibration was linear to less than 10 µg/g, but no detection limit was reported. — 25

* Colorimetric determination of blue-green color of Ni(II)-NTA chelate. Automatic determination with Technicon Auto-Analyzer in detergent formulations. — 26

* Colorimetric determination of Fe-NTA chelate in activated sludge effluents at concentrations between 5 and 50 mg/liter using the phenanthroline method. — 27

Potentiometric titration

* Potentiometric titration with Fe(III). Determination of EDTA, NTA, and DTPA (diethylenetriaminepentaacetic acid). Allows determination of 1 to 2% NTA in EDTA. — 28

* Potentiometric titration with Tl(III) and Cu(II). Determination of NTA in water and sewage. The lower detection limit was 100 µg/liter of NTA. The relative standard deviation in sewage samples was 5%, and the recovery varied from 90 to 110%. — 29

Atomic absorption spectroscopic technique

* Compleximetric atomic absorption spectroscopic method based on the formation of a soluble Pb-NTA chelate. — 30

Freezing of water samples following sterilization by filtration (Whatman GF/C, 2 to 3 μ pore size) was reported by Murray.[5] Water samples containing 20 to 2,000 μg/liter NTA were filtered, acidified with HCl and kept frozen in screw-capped bottles until analyzed. No change in NTA concentration was reported for samples kept frozen up to 6 weeks. NTA in unfiltered lake water was found to degrade in about 8 days at an unspecified temperature.[7] If, however, the sample was filtered through a 0.45 μ membrane filter, NTA was found to be stable for at least 20 days in Pyrex and polyethylene containers without deterioration.[7] Therefore, samples should be filtered immediately after collection if storage at room temperature is anticipated.

Stolzberg[14] reported that river water samples spiked with NTA at 1 to 50 mg/liter and incubated at room temperature under normal fluorescent lighting showed constant concentration of NTA for 1 to 3 days, after which undetectable levels of NTA were found.

A vial pretreatment procedure was investigated by Stolzberg.[14] Results in Table 2 indicate that vial pretreatment is unnecessary when handling milligram quantities of NTA. Simple washing with distilled water and drying is sufficient. However, when handling microgram quantities of NTA, it is especially important to preleach the reaction vials and to include ammoniacal EDTA masking reagent in the procedure, since NTA may complex with alkali and alkaline earth metal ions from glass.

TABLE 2. EFFECT OF VIAL PRETREATMENT AND MODE OF INTRODUCTION
μg OF NTA RECOVERED FROM 10 μg SAMPLES[a]

Vial pretreatment	Introduction technique[b]				
	A	B	C	D	E
None	0.0	0.0	1.9	7.1	10.0
Water washed	0.3	6.0	2.7	8.8	9.9
0.1 M HCl soak	1.7	6.9	6.0	8.9	7.7
0.1 M EDTA soak	2.7	0.0	2.9	9.3	10.0

a Data taken from Reference 14.
b Conditions:
　　A - Sample added as 1.0 ml of 10 μg/ml NTA.
　　B - 1 ml water evaporated in vial, then
　　　　10 μl of 1 μg/ml NTA added.
　　C - 1 ml 10 μg/ml NTA + 1 drop conc. HCl.
　　D - 10 μl 1 μg/μl NTA.
　　E - 1 ml 10 μg/ml NTA + 1 ml 150 μg/ml
　　　　EDTA.

Procedures for preservation of sediments for organic analysis include freezing or sterilization by autoclaving. Addition of acid, such as formic acid, that may inhibit bacterial activity for short period of time until

analysis may also be applicable. No information dealing with analysis of NTA in sediments is available; therefore, preservation procedures for sediments need to be established.

NTA ISOLATION FROM SAMPLE MATRIX

Reported techniques for concentration of NTA from water, include ion exchange columns, freeze drying, and evaporation. Following is a brief review of each of these techniques.

Ion Exchange Columns

Table 3 summarizes the type of ion exchange resins and their characteristics considered important in the concentration process. In order for exchange to take place, the solution containing the compound of interest, in this case NTA, is passed through the resin and the resin counterions are replaced by the sample ions of like charge. Neutral molecules and those having the same charge as the resin functional groups pass through the resin. Following cleanup with small volumes of 0.1 M formic acid to wash-off other interferences, 16 M formic acid is then used to elute the sorbed ions. Formic acid is then removed completely from the sample prior to derivatization.

TABLE 3. ION EXCHANGE RESINS

Resin	Base	Mesh	Exchange group	Exchange capacity (ECV) (meq/mL)	Form
Dowex 1-X8	Styrene-divinylbenzene	50/100	$CH_2{}^+N(CH_3)_3$	1.4	$HCOO^-$ Cl^-
Bio-Rad AG1-X2	Styrene-divinylbenzene	50/100	$CH_2{}^+N(CH_3)_3$	0.7	$HCOO^-$

Several studies which used ion exchange resins to concentrate NTA from water will be briefly described below.

Davies[31] concentrated a number of carboxylic acids from water using an ion exchange column and found that NTA can be concentrated on Dowex-1 resin in the formate form and eluted with 5M HCOOH. Recovery of NTA by elution with HCOOH of different molarities and the effect of pH on recovery of NTA using Dowex 1-X8 resin was reported by Chau.[7] Results indicate that NTA can be quantitatively retained by resin at pH 2.5 to 10 (see Table 4). Recovery data of NTA by elution from resin with HCOOH of different molarities (1 to 8 M) indicate quantitative recovery with acid molarities > 2 M.

TABLE 4. % RECOVERY OF NTA BY CONCENTRATION
ON DOWEX 1-X8 RESINS

Elution with HCOOH			Effect of pH	
Acid molarity	Volume (ml)	Recovery (%)	pH	Recovery (%)
1	100	56	1	0.3
2	75	96	2.2	90
2.5	75	99	2.6	101
3	75	99	3.5	99
4	75	101	5	102
5	50	102	6	100
6	50	101	8	101
7	50	99	8.6	102
8	50	101	9.5	103
			10.3	102

Aue[8] used a similar resin, Bio-Rad AG1-X2 (different percentages of crosslinking agent), to concentrate NTA. A resin with a lower percentage of crosslinking has an open structure which is more permeable to higher molecular weight substances than a high crosslinking resin. It also swells more when in use.

Despite the fact that ion exchange columns are recommended to concentrate NTA from water, no systematic study of the concentration process was reported. Most studies recommend use of 50 mm length x 5 mm ID resin bed for a 50 to 100 ml volume of water sample. Before runs, 16 M or 25 M formic acid is passed through the column, followed by deionized water. The sample is poured into the reservoir and allowed to flow at 3 ml/min. Following the sample, 0.1 M formic acid is used to wash off the impurities and NTA is eluted from the column with 16 M formic acid.[8,9] Malaiyandi[9] used this procedure to analyze for NTA in drinking water and reported recoveries > 90% at concentrations ranging from 1 to 25 µg/liter of water. Games[12] reported that use of 0.1 M HCOOH to wash off interferences prior to elution of NTA led to positive interferences which had a significant effect upon the recovery of NTA. Use of 2 M HCOOH (10 mL) for initial cleanup and 8 M HCOOH (10 ml) to elute NTA gave good and reproducible recoveries. Figure 1 is a series of chromatograms that show the effect of removing positive interferences in order to improve the chromatography of NTA. Each chromatogram was obtained on the same sample, which was subjected to different cleanup and eluted with a different concentration of HCOOH. NTA recovery was > 95% in A through C and 67% in D. The concentrations of formic acid used were A, 0.1 M and 16 M; B, 1 M and 10 M; C, 2 M and
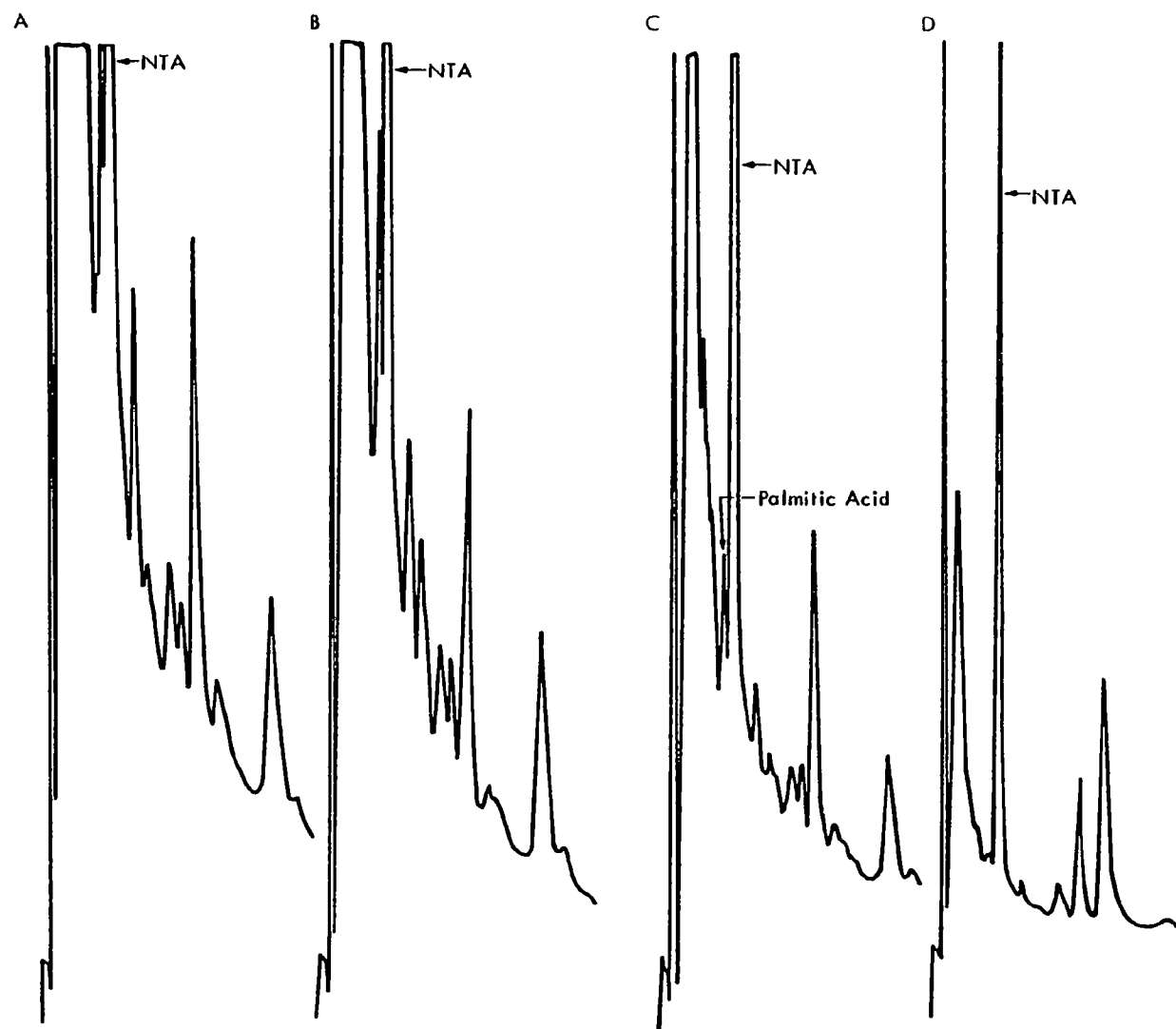
Figure 1 – GC/NPD chromatograms showing the effect of removing positive interferences to obtain a clean separation of NTA – the concentrations of HCOOH used were: A. 0.1 M and 16 M; B. 1 M and 10 M; C. 2 M and 8 M; D. 4 M and 8 M. Taken from Reference 12.

8 M; D, 4 M and 8 M. Results of the validation study using samples from various locations in the USA by the Games procedure,[12] slightly modified to allow correction of recovery using ^{14}C-NTA, are given in Table 5. It can be noted that relative standard deviations are about 10% at concentrations ranging from 1 to 50 µg/liter. Corrected NTA recoveries vary from 77 to 114%.

TABLE 5. RESULTS OF THE VALIDATION STUDY FOR THE
MODIFIED METHOD USING SAMPLES COLLECTED FROM
VARIOUS LOCATIONS IN THE USA[a]

	NTA added (µg/liter)	No. of samples	% Recovery
Influent sewage	50	7	114 ± 10
Effluent sewage	10	8	106 ± 17
River water	5	5	77 ± 11
Tap water	1, 2, 3	9	88 ± 10

a Data taken from Reference 12.

Freeze Drying

Murray used freeze drying or lyophilization to concentrate small volumes of samples (10 ml). Prior to lyophilization, 0.1 ml concentrated HCl was added to the sample. The ampules were shell-frozen in a dry-ice acetone mixture and lyophilized on a freeze dryer. Lyophilization is applicable to all water types and allows the separation of precipitated inorganic salts and by redissolving the residue in an organic solvent. However, the technique is very time consuming if larger volumes of water have to be handled. No recovery data was available.

Evaporation

Evaporation of water samples in a porcelain dish at 115°C in a drying oven or at 150°C in a sand bath under a moderate stream of nitrogen was reported.[6] The residue was rinsed with 16 M formic acid, and the formic acid was completely evaporated by heating the flask in a sand bath at 110°C under nitrogen. No recovery data were reported.

Solvent Extraction

When NTA is present in a solid matrix, such as river sediment, use of any of the techniques described above is not possible. Extraction from the matrix with water or a dilute aqueous base might be possible since NTA is

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very soluble in water. Once extracted in an aqueous solution or an organic solvent, the compound can be treated as a water sample as described above. No data were found on the partition ratio of NTA between water and sediment, so the probability that NTA is present in sediment cannot be predicted.

CHEMICAL DERIVATIZATION

Techniques available to derivatize NTA prior to gas chromatographic analysis are reviewed. Derivatization is done to reduce compound polarity, and to increase is volatility and its chemical stability. Derivatization, however, is quite often avoided because it can seriously affect the precision and accuracy of the method if not performed properly. Furthermore, derivatization may be a potential source of contamination if reagents used are not pure or may lead to undesirable by-products or artifacts. Since NTA is not volatile, gas chromatographic analysis requires prior esterification. Table 6 summarizes the reagent procedures and the detection systems reported for NTA. Each derivatization technique will be discussed briefly below.

Rudling[4] developed a method based on the derivatization of NTA in the presence of EDTA using BF_3 in methanol (10% w/v). Concentrations lower than 200 µg/liter of NTA were difficult to analyze owing to the poor chromatographic properties of the trimethyl ester of NTA.

Murray[5] used methanol/HCl and dimethyl sulfite to derivatize NTA to its trimethyl ester. Esterification was found to be at its maximum after 3 hr at 68°C. Shorter esterification times gave peaks that were thought to be mono- and dimethyl esters of NTA.

Esterification of NTA with n-propanol/HCl was reported by Chau.[7] Since n-propanol refluxes efficiently at 100°C, this temperature was recommended. Esterification times between 30 min and 1 hr were found to give complete reaction over the range of 1 to 20 µg.

Reichert[6] used n-propanol/acetyl chloride to esterify NTA to its propyl ester. The derivatizing reagent in this case is the HCl-saturated n-propanol which forms by mixing n-propanol with acetyl chloride in a ratio 10:1. The propyl acetate which forms as by-product does not interfere with the esterification. By use of n-propanol/acetyl chloride, quantitiative esterification of NTA occurs within 30 min, and NTA is easily soluble in this mixture at the boiling point.

Several papers[8-13] report derivatization of NTA to its tributyl ester using n-butanol/HCl. The esterification reaction was efficiently optimized by use of a factorial experimental design.[13] Two HCl concentrations, two reaction temperatures, and two reaction times were investigated. The results are given in Table 7. The optimum conditions were found to be at: acid concentration 3 N, temperature 65°C, and reaction time 45 min. An increase in temperature to 150°C caused the cleavage of NTA to iminodiacetic acid (IDA). Longer reaction times also cause conversion of NTA to IDA.

TABLE 6. DERIVATIZATION METHODS USED FOR NTA

Derivative	Reagent	Procedure	Detection system	Reference
tri-Methyl ester	BF$_3$ in methanol 10% (w/v)	Evaporate sample to dryness Add esterifica- tion reagent (1 0 ml), seal ampule and place in an ultrasonic batch at 100°C for 40 min After cooling, add chloroform (1 ml) and trans- fer to a tube containing 3 ml buffer solution (NaOH/K$_2$HPO$_4$, pH = 7) Shake 1 min and remove chloroform layer.	GC/FID	4
	Methanol/HCl and dimethylsulfite	Evaporate sample to dryness Add 1 ml MeOH/ HCl and 0 2 ml dimethylsulfite. Seal vials, place in water bath at 68°C for 3 hr Remove excess reagents on a rotary evaporator	GC/FID GC/MS	5
tri-Propyl ester	n-Propanol/acetyl chloride (10 1)	Evaporate sample to dryness Add 2 ml n-propanol/acetyl chloride Heat at 110°C for 30 min in a sand bath Heat mixture at 110°C for 10 min while passing N$_2$ through to evapo- rate solvent to 1 ml Cool Add 0 5 ml methyl- cyclohexane and 2 ml 1 N NaOH Separate phases	GC/NPD	6
	n-Propanol/HCl	1 ml Aliquot of NTA (10 µg) evaporated to dry- ness at 90 to 100°C Add 2 ml n-propanol saturated with HCl Reflux for 30 min	GC/FID	7
tri-n-Butyl ester	n-Butanol/HCl	2 ml n-Butanol/3N HCl is added to the dry sample Cap tightly and maintain 25 min in an ultra- sonic bath at 75°C	GC/FID GC/NPD GC/MS	8 9 10 11 12 13

(continued)

TABLE 6. (concluded)

Derivative	Reagent	Procedure	Detection system	Reference
tri-Trimethylsilyl ester	Bis(trimethyl-silyl)trifluoro-acetamide (BSTFA)	Mask interfering cations with EDTA (2 mg EDTA per ml sample) Evaporate to dryness (presence of moisture would cause hydrolysis of TMS deriva-tive). Add 0 2 ml BSTFA and 0 2 ml DMF and agi-tate Heat sealed vial at 70°C for 30 min, cool Pyridine or acetonitrile can be used instead of DMF	GC/FID	14 15 16
tri(2-Chloroethyl) ester	Boron trifluoride in 2-chloroethanol 10% w/v	Evaporate sample to dryness at 100°C Add 1 ml esterification reagent, seal ampule, place in an oil bath for 2 hr Dissolve in benzene, wash with water	GC/EC GC/MS	17

TABLE 7. ESTERIFICATION OF NTA: ASSIGNMENT OF FACTORS[a]

Factors	Low level	High level
Acid concentration	1.25 N	3 N
Reaction temperature	65°C	100°C
Reaction time	10 min	45 min

a   Data taken from Reference 13.

Games[12] reported that residual HCl left in the vial after evaporation contributes to rapid loss in the performance of the nitrogen selective detector. Therefore, 1 M HCl was used, and the temperature was increased to 85°C. Final traces of HCl/butanol were removed by adding methanol to the dried sample and immediately evaporating it at 85°C.

Stolzberg[14] reported derivatization of NTA to its trimethylsilyl derivative. The sample is evaporated directly in the reaction vial with an excess of the ammonium salt of EDTA to mask interfering cations and to connect NTA to a more volatile compound. To efficiently remove water from the vial, addition and evaporation at 70°C of two portions of dry methylene chloride is recommended. The importance of complete removal of water is demonstrated by the fact that 1 mg of water can consume approximately 20 mg of BSTFA reagent. Effects of vial pretreatment with microgram amounts of NTA was investigated, since leaching of alkali and alkaline earth metal ions from the glass was thought to cause incomplete derivatization. The results, summarized in Table 2, show that preleaching of the reaction vials and masking the interfering cations with ammonium salt of EDTA are required in order to avoid erratic results.

To increase the sensitivity of the detection technique derivatization of NTA to its tri(2-chloroethyl) ester and electron capture detection was reported.[17]

GAS CHROMATOGRAPHY

All gas chromatographic techniques reported in the literature require derivatization of NTA to its volatile ester. Derivatization techniques were discussed in the preceeding section. A list of packing materials that have been used to chromatograph the ester derivatives is given in Table 8.

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TABLE 8. PACKING MATERIALS REPORTED FOR ANALYSIS OF
NTA ESTERS BY GAS CHROMATOGRAPHY

GC packing material	GC conditions	Reference
5% OV-17 on Aeropak (100/120 mesh)	150-285°C at 10°C/min	4
2% Ethylene glycol adipate on Chromosorb W	isothermal at 195°C	5
2% FFAP on Chromosorb W	isothermal at 195°C	5
3% OV-1 on Chromosorb WHP (80/100 mesh)	isothermal at 195°C	6
3% OV-1 on Chromosorb WHP (80/100 mesh)	180-225°C at 3°C/min	7
Carbowax 20M on Celite 545 (100/120 mesh) (similar to 0.3% Carbowax 20M on acid washed Chromosorb W)	isothermal at 183°C	8
5% OV-101 on Chromosorb WHP (80/100 mesh)	isothermal at 235°C	10
3% OV-210 on Chromosorb WHP (80/100 mesh)	isothermal at 200°C	
3% OV-210 on acid washed Chromosorb W (60/80)	145°C (8 min) to 240°C at 6°C/min	11
Ultrabond 20M (100/200 mesh)	215°C (8 min) to 250°C at 20°C/min	12

The possibility that other compounds may interfere in the analysis of NTA was investigated.[5] The esters of saturated, unsaturated, and branched chain fatty acids were chromatographed with the NTA trimethyl ester. Interference was found with the C_{18} iso ester. Possible breakdown products of NTA such as methyliminodiacetic acid (MIDA) and iminodiacetic acid (IDA) were reported to have shorter retention times then the trimethyl NTA and would not interfere in the analysis.

Several detectors, including flame ionization detector (FID),[4,5,7,11,13-16] nitrogen selective detector (NPD or TSD),[6,9,10,12] electron capture detector (ECD)[17] and mass spectrometer (MS),[5,10,17] have been used to detect the NTA esters. Both internal standard and external standard quantitation were reported. Nitrilotripropionic acid was used as internal standard by Games. A set of three standards at concentrations similar to those expected in the samples were run each day to establish the standard curve. The response to NTA using NPD was linear from 1 µg/liter to 5,000 µg/liter.

Detection of NTA using an element selective detector such as NPD has a higher degree of reliability over FID, however confirmation of NTA by GC/MS is preferred. MS can be used at the same time to quantitate the samples. A comparison of quantitation using NPD and GC/MS in the selected ion monitoring mode (SIM) was reported operating the mass spectrometer in both the electron impact (EI) and the chemical ionization (CI) mode. Ammonia was used as the CI gas. Ions selected for EI were m/e 158 and m/e 258 (see mass spectrum in Figure 2). For CI, ions monitored were at m/e 360 (M + 1) and m/e 246. Table 9 shows a comparison of GC and GC/MS/SIM data for four sewage samples (both influent and effluent). Analyses were done on the same extract after derivatization so that variations in recovery would not affect the comparison. Although daily fluctuations of the NPD response were reported, no solution was found. It was recommended that a standard curve be obtained with each set of four to five samples. Use of an internal standard of similar structure, such as nitrilotripropionic acid (NTP) resulted in improved consistency of the response factors over the concentration range of interest. Methanol was preferred over acetone as the solvent since the tributyl ester of NTP was not sufficiently soluble in acetone. It is also recommended[12] that sample extracts that had been derivatized and were ready to be analyzed for NTA, should be stored dry at room temperature and addition of methanol should be done immediately prior to analysis.

HIGH PRESSURE LIQUID CHROMATOGRAPHY

Analysis of NTA in sewage samples by high pressure liquid chromatography using a strong anion exchange resin coated onto Zipax and 0.02 M $Na_2B_4O_7 \cdot 10 \ H_2O$ as the mobile phase was reported.[18] Possible interferences from metallic ions were overcome by converting all metal-NTA chelates to ferric chelate. Following addition of the ion salt, 100 µl of the water sample was injected directly onto the column. The column effluent was monitored at 254 nm. Although the utility of this method has been demonstrated using both sewage samples and detergent formulations, additional confirmation of NTA is required. For positive identification the hydrolyzed chelate had to be further purified and confirmed by both infrared and gas chromatographic techniques. Furthermore,
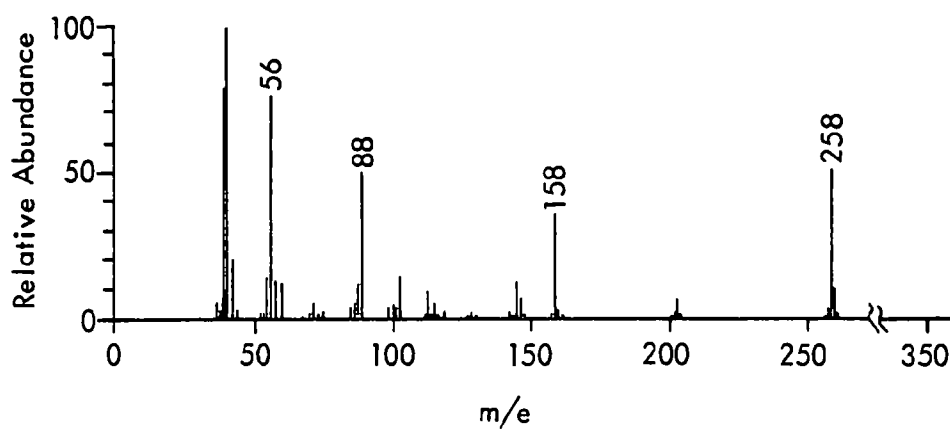
Figure 2 - Electron impact mass spectrum of tri-n-butyl ester of NTA.
The molecular ion, m/e 350, can be detected if the
spectrum is magnified ca. 10x.

TABLE 9. COMPARISON OF GC AND GC/MS/SIM RESULTS FROM
SEVERAL SEWAGE SAMPLES USING GAMES' METHOD[a]

Sample type	GC quantitation (µg/ℓ) (NPD detector)	SIM/GC/MS quantitation (µg/ℓ)
Sewage influent		
A	16	14
B	6	10
C	52	56
D	13	10
Sewage effluent		
A	3	5
B	< 1	0
C	6.1	11
D	1.2	0

a Data taken from Reference 12.

method sensitivity is only 1 mg/liter and the response is linear only through 0.1 to 1.5 µg range. Beyond 1.5 µg the exchange capacity of the column was exceeded and the NTA eluted early. At lower levels, peaks were quite broad and difficult to measure. Because of this, the precision of the method was quite poor. At 1 µg level the relative standard deviation was 2%, whereas at 0.2 µg the relative standard deviation was 25%.

OTHER ANALYTICAL TECHNIQUES

As briefly described in Table 1, there are various other analytical techniques, such as polarography, colorimetry, and potentiometric titration, that can be used to analyze for NTA. They are all based on the complexing ability of NTA. Some of these methods (e.g., colorimetric, potentiometric titration) lack the sensitivity and the selectivity required to detect trace levels of NTA in environmental samples. Nonetheless, they were quite popular in the late 1960's due to their simplicity and the widely accepted reliability of these colorimetric techniques. Following is a review of these techniques. Polarographic techniques will be discussed first, followed by colorimetric and potentiometric titration methods.

Polarographic methods are based on the reduction of a suitable metal ion complexed with NTA. Since the half-wave potentials for the reduction of complexes of the same ion vary appreciably with the ligand, the procedure is almost as specific for NTA as the GC methods. Daniel and LeBlank[32] described a polarographic method for the analysis of NTA in presence of EDTA. A cadmium complex was formed by addition of excess cadmium salt. The free Cd^{2+} and the Cd-NTA complex give two well resolved polarographic waves. The half-wave potential is -0.97 V for Cd-NTA complex, -0.6 V for Cd^{2+}, and -1.4 V for Cd-EDTA complex. This method was used by Asplund[19] to analyze samples of lake water containing NTA at concentrations from 1 to 10 mg/liter.

Afghan[20] used differential cathode ray polarography to detect NTA as "free" NTA and as "total" NTA at levels as low as 10 µg/liter without any sample preconcentration. "Free" NTA in natural water is determined using lead as reagent and pH 8 and varying the potential from -0.5 V to -1.0 V versus mercury pool reference electrode. The Pb-NTA complex has a half-wave potential at -0.9 V. Determination of "total" NTA is based on releasing NTA from metal complexes under acidic conditions. Addition of EDTA and adjustment of pH to 8 will preferentially bind the metals with EDTA. NTA is then measured as indicated above by forming the Pb-NTA complex. Interferences were found from ethylene glycol-bis(p-aminoethylether)-N,N-tetraacetic acid (EGTA) and tripolyphosphate which have half-wave potentials very close to Pb-NTA complex.

Afghan[21] also reported an automated method using dc polarography for the determination of NTA in waters, sewage, and detergents. This method is based on the formations of Bi-NTA complex at pH 2 and has a sample throughput of 15 samples/hr. A detection limit of 10 µg/liter and a coefficient of variation at 100 µg/liter of 1.3% was reported. Interference from other compounds including amino acids, K_2HPO_4, glucose, nutrient broth, peptone, urea, etc. was investigated.

Another differential polarographic method was reported by Haberman[22] using an In(III)-NTA complex. The technique was evaluated with river water and sewage samples containing NTA at levels between 26 μg/liter and 2.6 mg/liter. ^{14}C-NTA was added to samples to correct for incomplete recovery. No interference was found in distilled water with a tenfold molar ratio of EDTA, citrate, maleate, phosphate, carbonate or sulfate to 2.6 mg/liter Na_3NTA. One disadvantage of this technique was the high concentration of indium required for complete NTA recovery. At a mole ratio of 5:1 of In-NTA the polarographic peak of In-NTA complex is not well resolved from the indium reduction peak.

Taylor et al.[16] evaluated polarography using Pb-NTA, Cd-NTA, and Bi-NTA complexes to determine which gave the best defined polarographic peaks. Pb-NTA complex was investigated in three buffer systems: tris-hydrochloride-ammonium hydroxide, glycine-ammonium hydroxide, and ammonium chloride-ammonium hydroxide, at pH 7-8. Cd-NTA complex was measured in ammonium chloride-ammonium hydroxide buffer at pH 9. Bi-NTA complex was investigated in NaCl media at pH 2. Polarographic determination of NTA as the Bi-NTA complex was found to be the most sensitive (50 μg/liter detection limit).

More modern polarographic techniques such as differential pulse polarography have been evaluated by Hoover[23] and Stolzberg.[24] Differential pulse polarography is more sensitive than dc polarography and can be used to measure small currents at a potential cathodic of an electrochemically active species present at much greater concentration. Both Bi-NTA complex[23] and Cd-NTA complex[24] were investigated. Relative standard deviations of < 5% were reported by Hoover[23] at 1 mg/liter levels of NTA and calibrations were linear from 10 μg/liter to 4 mg/liter. An acetate electrolyte was much better than chloride electrolyte for Bi-NTA complex in sewage samples.

Colorimetric methods for NTA analysis are based on the measurement of the absorbances of the colored metal chelates that are formed with excess metal ions. For example, a method by Tabatai[25] measures the blue color obtained by treating the sample with borate buffer (pH 9.2), zinc sulfate and 2-carboxy-2'-hydroxy-5'-sulfoformazylbenzene (Zincon). Vanwelssenaer[26] measured the absorbance at 600 nm of the blue-green color of the Ni-NTA chelate, and Swisher[27] developed a method that utilizes the formation of a stable 1:1 complex of NTA with ferric ions and is measured at 502 nm. Although rapid, colorimetric techniques are not very sensitive (mg/liter sensitivity) and are subject to interference by other chelating agents. Colored material in samples can also interfere.

Another instrumental method used is potentiometric titration with iron (III) chloride,[28] thallium (III) nitrate[29] and copper (II) nitrate.[16,29] The titration curves (mV versus ml titrant) consisted of two equivalence points, the first belonging to thallium (or copper) consumed by ammonium pyrrolidine-dithiocarbamate (APDC) and the second end point belonging to NTA or other ligands in the sample. The lowest concentration for titrants was 10^{-4} M. Since metal cations were present in sewage usually as complexes with NTA, the sample was first titrated at pH 5.5 to eliminate Ca, Mg, Zn, Ba, followed by precipitation and filtration of hydroxides at strongly alkaline pH, and addition of APDC which precipitated cations in the presence of NTA. The excess of APDC was titrated with Ti^{3+} and Cu^{2+}. The detection limit was 0.1 ppm.[29]

Although potentiometric titration techniques offer somewhat greater sensitivities than colorimetric techniques, they are elaborate and time-consuming.

A compleximetric atomic absorption spectrometric (AAS) method based on the formation of a soluble Pb-NTA chelate that is determined by AAS was reported.[30] Pretreatment of sample was minimal requiring only pH adjustment and dilution. Matrix effects were counteracted by using the method of standard additions.

SECTION 5

NTA MONITORING DATA

CANADIAN DRINKING WATER STUDY

The program for monitoring NTA in Canadian waters began in 1971 and was carried out by the Inland Waters Directorate, Fisheries and Environment, in Canada in cooperation with Procter & Gamble Company of Canada, Limited.[3] The purpose of this program was fourfold:

* Survey of NTA in the finished drinking water in municipalities across Canada (four annual samplings).

* Survey of NTA in raw water above and below 13 selected Ontario municipalities and the municipal tap water (monthly sampling).

* Study Hamilton Harbour, the western end of Lake Ontario, rivers in the metropolitan Montreal area, and several selected groundwaters.

* Survey NTA in the international section of St. Lawrence River.

The survey of NTA in finished drinking water program was undertaken by Canada Centre for Inland Waters (CCIW); the program to survey NTA in 13 Ontario municipalities was undertaken by Procter & Gamble Company in cooperation with the Ontario Ministry of the Environment and CCIW. The other two programs involved sampling over shorter periods of time than those covered by the monitoring program. No detailed sampling rationale or design was given. However, some of the special study sites were purposely selected on the basis of prior knowledge or prediction of the NTA discharge into the water system.

Two analytical methods were employed. CCIW used the polarographic method by Afghan[20] (detection limit 10 µg/liter) and Procter and Gamble used the gas chromatographic method by Warren[13] (detection limit 1 µg/liter). In both laboratories, the samples were preserved with formaldehyde. No other analytical details were given.

Table 10 summarizes the data for NTA showing the number of samples examined and the number of samples in which NTA was present at or above 10 µg/liter (method detection limit). It can be noticed that the largest number of samples were from Ontario and Quebec. The summaries of the Ontario and Quebec samples are given in Tables 11 and 12, respectively.

TABLE 10. NATIONAL MONITORING PROGRAM SAMPLES[a,b]

Province	Number of samples examined				Number of NTA positive samples			
	1972	1973	1974	1975	1972	1973	1974	1975
British Columbia	3	78	9	36	0	0	0	1
Alberta	6	7	6	7	0	1	1	0
Saskatchewan	6	8	5	7	0	2	0	2
Manitoba	6	8	7	7	0	1	0	0
Ontario[b]	76	76	73	60	3	1	3	4
Quebec	60	58	54	57	0	2	11	16
New Brunswick	5	10	0	5	0	0	0	0
Nova Scotia	6	15	0	9	0	0	0	0
Newfoundland	0	4	5	0	0	0	0	0
	168	264	159	188	3	7	15	23

a Polarographic method by Afghan[20] and gas chromatographic method
   by Warren[13] were used to analyze samples.
b Data taken from Reference 3.

TABLE 11. SUMMARY OF ONTARIO SAMPLES[a,b]

Great Lakes system	Number of samples examined				Number of NTA positive samples			
	1972	1973	1974	1975	1972	1973	1974	1975
Lake Superior to Detroit River	7	7	7	6	1	0	0	0
Lake Erie	4	4	6	4	0	0	0	0
Welland Canal	6	6	6	5	0	0	1	0
Niagara River	2	2	2	2	0	0	0	0
Lake Ontario	21	23	22	13	0	0	1	2
St. Lawrence River	2	2	2	2	0	0	0	0
Ottawa River	6	5	3	3	0	0	0	0
Small lakes	7	5	5	5	0	0	0	0
Small rivers	8	8	8	8	2	1	1	1
Groundwaters	13	14	12	12	0	0	0	1
	76	76	73	60	3	1	3	4

a Gas chromatographic method by Warren[13] was used to analyze samples.
b Data taken from Reference 3.

TABLE 12. SUMMARY OF QUEBEC SAMPLES[a,b]

Source	Number of samples examined				Number of NTA positive samples			
	1972	1973	1974	1975	1972	1973	1974	1975
St. Lawrence River*	4	4	4	4	0	0	0	0
Ottawa River*	2	2	2	2	0	0	0	0
Montreal area	15	14	13	13	0	1	8	6
Richelieu River	5	4	4	5	0	0	0	1
Yamaska River	3	3	3	3	0	1	2	2
Riviere l'Assomption	2	1	0	1	0	0	0	0
Small rivers	13	11	11	13	0	0	0	4
Small lakes	14	17	15	14	0	0	1	3
Groundwater	2	2	2	2	0	0	0	0
	60	58	54	57	0	2	11	16

* Excluding Montreal area.
a Polarographic method by Afghan[20] and gas chromatographic method by Warren[13] were used to analyze samples.
b Data taken from Reference 3.

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Seventy-eight samples of municipal drinking water samples collected from groundwater from four Canadian provinces during the 4 years of the national monitoring program were also monitored for NTA. Results given in Table 13 indicate that deeper aquifers are not likely to contain significant amounts of NTA. However, when municipal groundwaters were analyzed for NTA (see data in Table 14) 90% of the samples were found to contain 5 µg/liter or less and the median value was 0.5 µg/liter.

The other studies (e.g., Hamilton Harbour, Lake Ontario, St. Lawrence River, etc.) showed much lower levels of NTA due to environmental degradation. The Hamilton Harbour, for example, can be considered as a completely mixed reactor with an input of 100 to 125 µg/liter, a retention time of 15 months, and an effluent with an average concentration of 15 µg/liter. Likewise, the St. Lawrence River, under the extreme conditions of the total NTA usage by 10 million people and allowing for no degradation in the wastewater treatment or in the river, the NTA concentration would be about 30 µg/liter with a more realistic figure of 10 µg/liter or less.[3]

Despite the considerable amount of data collected under this study, there was difficulty in interpreting the data because of the difference in values reported on comparison samples analyzed by the two laboratories. Furthermore, the polarographic method used by CCIW was sensitive to only 10 µg/liter and, therefore, most locations were reported as showing no detectable levels of NTA.

Malaiyandy et al.[9] has continued the study, sampling 70 municipalities across Canada. The sampling locations are given in Table 15. Grab samples were taken at each public water supply system from the raw water influent at the immediate vicinity of the water treatment plant (sample A), treated water samples (sample B), treated water samples from stations at 0.5 to 1.0 mile from the treatment plant (samples C and D). Further consideration was given to groundwater sources, disinfection methods, and major and minor drainage basins.

The sampling locations were selected to cover a statistically significant representation of population density in Canada. Nineteen private homes and two commercial establishments in the Ottawa-Carleton Region, Boulbourn and Lanark were also sampled. All samples were collected during winter months from November 1976 to February 1977. A gas chromatographic method with detection limit of 0.2 µg/liter was used to analyze these samples.

Data shown in Table 15 is summarized in Table 16 where the mean values of NTA from all sources are given. The highest levels of NTA were found in the St. Lawrence and Great Lakes basins which receive higher levels of NTA compared to other basins.

A comparison of the levels of NTA found in 10 municipal water surveyed prior to 1975[3] and in 1976-1977[9] is shown in Table 17. Examination of the data indicates that NTA levels have decreased in five municipalities and remained the same in other five. Investigation of the various treatment processes at these locations (prechlorination, pre- and postchlorination, and ozonation) concluded that none of the treatment processes appears to be completely effective for NTA removal.

TABLE 13. GROUNDWATER SAMPLES[a,b]

Province	Number of samples examined				Number of samples containing NTA (10 ppb or > 10 ppb)			
	1972	1973	1974	1975	1972	1973	1974	1975
British Columbia	0	11	1	4	0	0	0	0
Saskatchewan	1	1	0	1	0	0	0	0
Ontario	13	14	12	12	0	0	0	0
Quebec	2	2	2	2	0	0	0	0
	16	28	15	19	0	0	0	1

a Polarographic method by Afghan and gas chromatographic method by Warren[13] were used to analyze samples.
b Data taken from Reference 3.

TABLE 14. NTA IN MUNICIPAL GROUNDWATERS[a,b]

Area	Population	Number of samples	Number of samples in range				Maximum (ppb Na_3NTA)
			0-1	2-5	6-10	10	
Frankford	1,600	29	20	7	2	0	8
Guelph	51,000	29	20	5	2	2	12
Kemptville	2,000	29	13	11	5	0	9
Stouffville	3,200	29	22	7	0	0	5
Stratford	23,000	29	20	7	2	0	8
Uxbridge	2,300	29	22	3	1	3	36
Number of samples		174	117	40	12	5	

a Polarographic method by Afghan[20] and gas chromatographic method by Warren[13] were used to analyze samples.
b Data taken from Reference 3.

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TABLE 15. LEVELS OF NTA FOUND IN THE 70 MUNICIPALITIES ACROSS CANADA[a]

City	Province[b]	Population served	Levels of NTA, $\mu g/\ell$[c] A	B	C	D
Barrie	ON	30,756	+[d]	+	+	+
Bathurst	NB	16,674	0.7	0.6	0.5	0.5
Belleville	ON	35,507	9.2	13.9	7.0	5.6
Brandon	MN	36,000	0.8	0.3	0.5	0.3
Brantford	ON	70,000	14.7	11.5	16.7	24.5
Calgary	AL	457,112	1.0	1.6	...[e]	1.2
Cap-de-Madelaine	QU	33,000	+	+	+	+
Cayuga	ON	1,070	33.5	30.4	15.4	7.4
Charlottetown	PE	19,500	0.5	+	0.3	+
Clarenville	NF	1,900	0.4	+	+	+
Dartmouth	NS	72,000	0.4	+	+	+
Dawson Creek	BC	18,000	0.3	+	+	0.3
Drummondville	QU	44,200	21.3	+	16.9	22.0
Edmonton	AL	530,000	1.6	1.7	1.8	2.0
Fredericton	NB	44,000	1.2	0.5	0.5	0.5
Granby	QU	34,000	18.9	0.3	+	+
Grand Falls	NF	9,153	+	+	0.3	+
Grand Prairie	AL	16,618	+	+	+	+
Halifax	NS	111,500	0.3	+	+	+
High Prairie	AL	5,000	+	0.7	0.9	0.7
Hudson	QU	4,500	...	+	+	+
Hull-Gatineau	QU	100,000	6.1	4.7	3.8	4.1
Kamloops	BC	28,000	+	...	+	+
Kelowna	BC	21,000	+	+	+	+
Lachine	QU	73,900	27.3	21.8	25.6	23.0
Laval	QU	250,000	14.3	12.6	13.0	11.8
Lethbridge	AL	45,000	3.4	2.3	4.3	3.3
Levis	QU	23,300	14.4	9.6	5.0	7.7
Longueil	QU	220,000	3.7	3.3	3.1	3.7
Magog	QU	15,155	0.8	+	+	+
Medicine Hat	AL	301,000	2.8	2.8	2.8	3.4
Moncton	NB	58,000	0.5	0.4	...	0.3
Montreal	QU	1,888,000	1.1	0.9	0.7	0.9
Nanaimo	BC	38,000	+	+	+	+
Niagara-on-the-lake	ON	3,670	1.2	0.9	0.7	0.9
North Vancouver	BC	41,000	+	0.4	0.3	+
Ottawa-Carleton	ON	442,000	2.5	2.1	2.3	2.5
Penticton	BC	50,000	+	+	+	+
Pierrefonds	QU	70,000	20.0	12.9	12.6	12.2
Portage la Prairie	MN	14,000	162.0[f]	101.3[f]	83.3[f]	124.4[f]
Port Hawkesbury	NS	4,000	0.4	+	+	0.5

(continued)

TABLE 15. (concluded)

City	Province[b]	Population served	Levels of NTA, $\mu g/\ell$[c]			
			A	B	C	D
Prince Albert	SK	30,000	5.6	...	7.6	5.6
Prince George	BC	45,000	+	0.3	+	+
Quebec	QU	225,000	3.9	3.5	2.7	0.3
Regina	SK	148,200	+	+	+	+
Rimouski	QU	32,716	0.8	0.4	0.5	0.4
Riviere du Loup	QU	15,000	6.2	...	1.9	...
St. Eustache	QU	5,000	11.7	...	11.4	13.3
Saint John	NB	103,000	+	+	+	+
St. Johns	NF	97,550	0.6	0.7	0.4	0.3
Saskatoon	SK	134,000	0.4	+	0.3	0.3
Selkirk	MN	10,000	+	+	+	+
Shawinigan	QU	44,000	+	+	+	+
Sherbrooke	QU	92,000	0.8	+	+	+
Smith Falls	ON	11,812	0.8	+	+	+
Sudbury	ON	91,500	0.5	0.4	0.4	0.3
Swan River	MN	4,000	7.9	12.8	8.0	8.1
Swift Current	SK	16,000	0.5	0.6	+	+
Thetford Mines	QU	26,000	0.6	0.6	0.5	0.4
Thunderbay	ON	102,529	1.5	0.5	0.4	0.6
Toronto	ON	2,000,000	1.6	4.8	4.4	4.7
Trois Rivieres	QU	60,080	3.4	2.8	3.1	2.0
Truro	NS	13,268	0.4	0.4	0.3	0.3
Vancouver	BC	1,000,000	+	0.3	+	+
Vermillion	AL	2,969	+	+	+	+
Victoria	BC	75,000	0.3	+	+	+
Windsor	ON	188,900	2.7	1.2	1.5	1.3
Windsor	QU	6,100	1.2	0.8	0.9	0.7
Winnipeg	MN	577,923	+	+	+	+
Yorktown	SK	14,500	0.5	+	+	+
Ottawa-Carleton[g]	ON	21 sites	...	16.9[g]	...	...

a Data taken from Reference 9; gas chromatographic method was used to analyze samples (method of detection 0.2 μg/liter).

b NF, Newfoundland; NS, Nova Scotia; PE, Prince Edward Island; NB, New Brunswick; QU, Quebec; ON, Ontario; MN, Manitoba; SK, Saskatchewan; AL, Alberta; BC, British Columbia.

c Symbols used are: A, raw water sample; B, treated water sample collected at treatment plant; C, treated water sample collected 0.5 mile from treatment plant; D, treated water sample collected 1.0 mile from treatment plant.

d The symbol + represents levels of NTA at 0.2 μg/liter or below.

e ..., sample unavailable.

f 1.0 μg/liter in repeat samples obtained in November 1977.

g Well water supplies; only one residence showed this level.

TABLE 16. MEAN CONCENTRATIONS OF NTA (in µg/liter) ESTIMATED
TO BE DISCHARGED INTO THE MAJOR DRAINAGE BASINS[a]

Major drainage basins	Raw H$_2$O		Finished H$_2$O	
	no. of cities sampled	mean	no. of cities sampled	mean
Atlantic Sea Board	12	0.5	12	0.3
Exploits	1	0.2	1	0.2
St. Lawrence	21	7.7	22	4.9
Great Lakes	9	7.2	9	5.8
Lake Winnipeg	14	1.6	14	2.1[a]
Mackenzie	3	0.2	3	0.4
Frazer	1	0.2	2	0.2
Columbia	2	0.2	2	0.2
Pacific Sea Board	4	0.2	4	0.2
	67	3.88	69	2.82

a Data taken from Reference 9.

TABLE 17. COMPARISON OF THE LEVELS OF NTA (in µg/liter) FOUND
IN TEN MUNICIPAL WATERS SURVEYED IN OR
PRIOR TO 1975 AND IN 1976-77[a]

Location	Province	Levels of NTA	
		in 1975 and before	in 1976-77
Granby	Quebec	80	0.2
Drummondville	Quebec	20	19.5
Shawinigan	Quebec	20	0.2
Sherbrooke	Quebec	10	0.2
Quebec	Quebec	20	1.9
Lachine	Quebec	20	23.5
Pierrefonds	Quebec	10	12.5
Brantford	Ontario	30	17.6
Brandon	Manitoba	82	0.4
Prince Albert	Saskatchewan	10	6.6

a Data taken from Reference 9.

The results of these two studies indicate relatively few locations where NTA levels occur above 10 µg/liter, and it was concluded that the use of NTA at that rate will not increase the levels of NTA in the major basins.

CANADIAN WASTEWATER STUDY

A long-term study of 13 Ontario cities measured NTA, nine metals and phosphorus in grab samples from sewage treatment plant influent and effluent and the receiving streams above and below the sewage outfall.[33] The monthly sampling from November 1971 to March 1975 spanned the changeover date in early 1973 when the NTA content of Canadian household detergents increased from 6% to 15%. This increased usage of NTA was reflected in the NTA content of sewage influent which increased from a median level of 1.3 mg/liter to 3.2 mg/liter. The sewage effluent also reflected the change in NTA usage, but less markedly. Following the change, the median level of NTA in the receiving stream below the sewage outfall was 0.05 mg/liter, with 97% of all samples containing less than 0.5 mg/liter. The levels were significantly higher (following changeover) in only 3 of the 13 locations.

OTHER STUDIES: REMOVAL OF NTA BY ACTIVATED SLUDGE

Several field studies to determine the removal of NTA during wastewater treatment have been reported.[34-36] Controlled dosages of NTA were added directly to the raw influent wastewater of an activated sludge plant unit, and NTA concentrations were monitored at various points within the plant and in the receiving system. Several conclusions were drawn from these studies.

* Average removal varied as a function of NTA concentration and type of treatment, for typical wastewaters being approximately 90% at dosages up to 8 mg/liter and about 75% at 16 µg/liter. Games[12] reported 90% removal of NTA at an average influent NTA concentration of 110 mg/liter when using activated sludge treatment and only 60-70% removal when using trickling filters (see Table 18).

* Removal of NTA by primary sedimentation is negligible (8%).[36] At pH values and NTA concentrations typical for wastewater (mg/liter range) adsorption of NTA on wastewater solids is negligible (< 2%)[36] due to its high water solubility and chelating properties. The 8% apparent removal reported in reference 36 may have been due to sampling procedure and not to adsorption on solids.

* The rate of NTA removal improved over a several month period.[37] This indicates that the efficiency of the sludge increases with acclimatization.

* Temperature appeared to be one of the most important factors in NTA degradation.[38] Table 19 gives the percent of NTA removal (concentrations 5-20 mg/ liter) at various temperatures using activated sludge.

TABLE 18   NTA DATA FROM FOUR TREATMENT PLANTS IN THE NETHERLANDS[a,b]

Location and plant type	Sample	Average temp	Average % influent recovery (range)	Average % effluent recovery (range)	Average NTA (µg/ℓ) influent	effluent	Percent NTA removal	Percent removal
Amersfoort Trickling	A	11 1	95(85-108)	92(86-99)	59	14	75	64
Filter	B	9 5	90(78-98)	105(97-114)	68	17	72	75
Soest baarn Trickling	A	12 6	93(74-108)	98(83-108)	61	21	63	76
Filter	B	9 8	75(63-90)	88(83-98)	64	20	61	75
Bunschoten Activated	A	11 4	84(60-105)	94(89-108)	53	7	86	82
Sludge	B	8 6	94(84-100)	84(76-91)	37	8	78	77
Harderwijk Activated	A	10 2	100(89-107)	95(90-104)	113	12	89	90
Sludge and	B	8 2	68(46-85)	94(87-100)	110	6	91	86
Tertiary Treatment								

a   The A samples were five consecutive 24-hr composite samples collected in November 1980, and the B samples were collected in December 1980
b   Data taken from Reference 12

TABLE 19. EFFECT OF TEMPERATURE ON REMOVAL
OF NTA UNDER STEADY CONDITIONS[a]

Temperature (°C)	% Removal of initial concentration	
	20 mg/ℓ	5 mg/ℓ
5	3	3
7.5	66	82
10	94	96
20	> 98	> 98

a Data taken from Reference 38.

It appears from data in Table 19 that while NTA is almost completely degraded during favorable conditions (temp. > 20°C), removal is incomplete during winter months. Similar temperature dependency of acclimated sewage has been reported both in a trickling filter and an oxidation pond[38] and also in an activated sludge treatment plant[40] where degradation was reduced significantly in winter. A more recent study showed that first order NTA degradation occurred over the temperature range studied, 2 to 24°C.[41] Kinetic data were given.

* Requirements for optimal biodegradation include moderate temperatures (> 20°C), prior and continued acclimation of sludge to NTA, aerobic conditions, and relatively low NTA loadings. NTA does not degrade under anaerobic conditions such as in septic tanks.[42] Acclimation of sludge to NTA generally required several weeks in field systems.[42] Temporary fluctuations or interruptions in NTA loadings produced prolonged reductions in degradation efficiency.[42]

* Possible pathways for biodegradation of NTA in sewage have been suggested (see Figure 3).[42] As can be seen, the first step in the degradation of NTA may involve a decarboxylation to N-methylnitrilodiacetic acid or deacetylation to iminodiacetic acid (IDA). N-Methylnitrilodiacetic acid can decarboxylate to dimethylglycine or demethylate to IDA. Dimethylglycine can also further demethylate to form N-methylglycine. Additionally, IDA can deacetylate to glycine. The possibility of accumulation of certain NTA intermediates and their nitrosation to form nitrosamines would have to be considered especially since nitroso-IDA forms readily at acidic pH. There are no available data on carcinogenicity testing of nitroso-IDA; nitroso-sarcosine is carcinogenic to rat.[42]

* Degradation of NTA was reported to continue in the receiving stream reaching 60% at 0.5 miles below outfall, and was essentially complete within 2 miles downstream.[36]
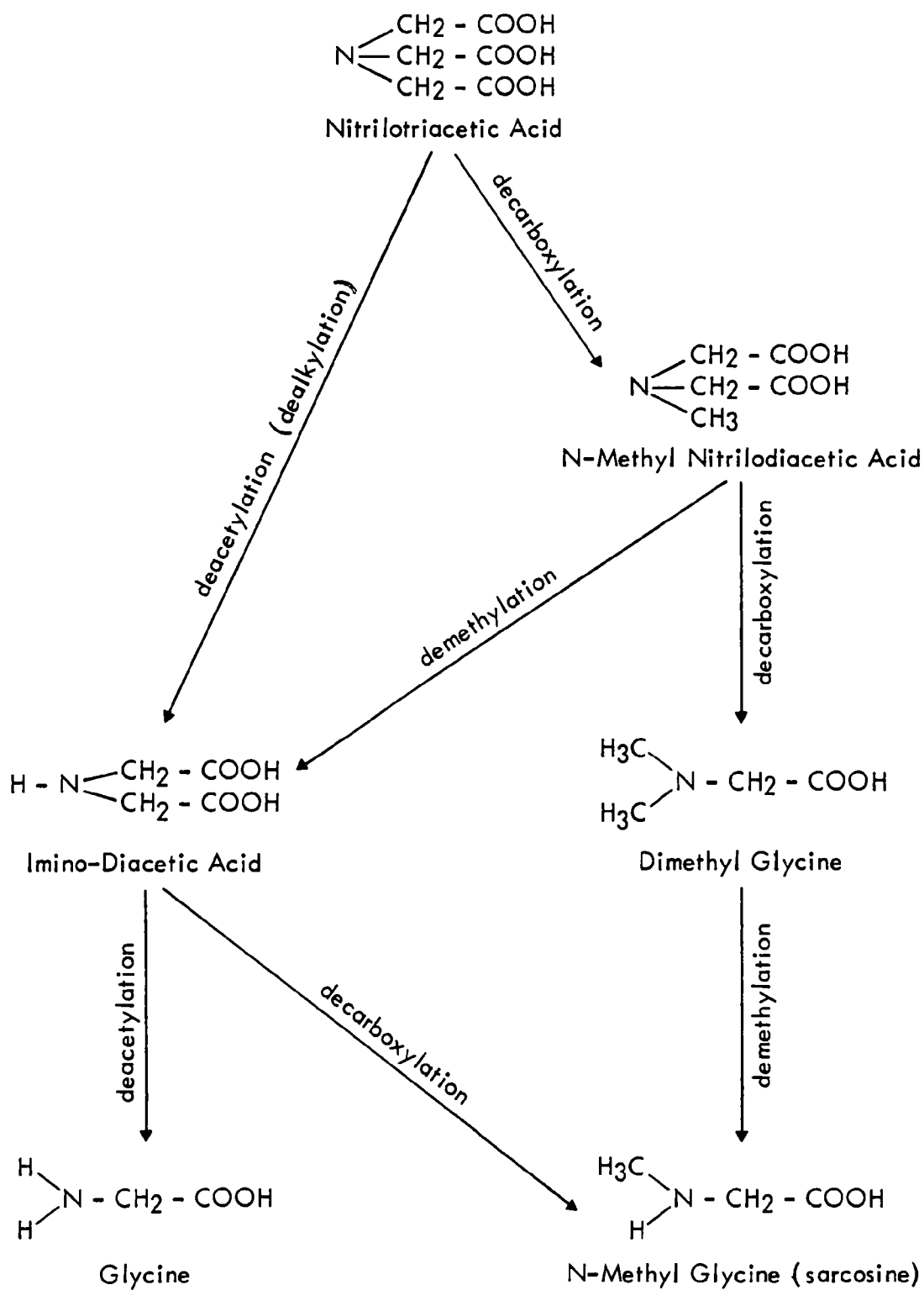
Figure 3 - Possible pathways for biodegradation of nitrilotriacetic acid in sewage. Data taken from Reference 40.

References

1. Farrow, R. N. P., and A. G. Hill, _Analyst_, <u>90</u>, 210 (1965).

2. Meiners, A. F., T. W. Lapp, C. E. Mumma, E. G. Podrebarac, R. P. Reidsdorf, H. R. Owens, and H. Gadberry, "Potential Worker and Consumer Exposures to Nitrilotriacetic Acid (NTA) in Detergents, EPA 560/11-79-008, June 1979, 45 pp..

3. Matheson, D. H., "Nitrilotriacetic Acid (NTA) in the Canadian Environment," _Sci. Ser. - Can., Inland Waters Dir._, <u>74</u> (1977), 46 pp.

4. Rudling, Lars, "Simultaneous Determination of Nitrilotriacetic Acid, Ethylenediaminetetraacetic Acid, and Diethylenetriaminepentaacetic Acid as Their Methyl Ester Derivatives by GLC (gas-liquid chromatography)," _Water Res._, <u>6</u>(7), 871-6 (1972).

5. Murray, D., and D. Povoledo, "Determination of Nitrilotriacetic Acid in Inland Waters by Gas Chromatography of the Trimethyl Ester," _J. Fish. Res. Bd. Can._, <u>28</u>(7), 1043-7 (1971).

6. Reichert, J. K. and A. H. M. Linckens, "A Simple and Sensitive Detection Method for NTA in Drinking Water and River Water," _Environ. Technol. Lett._, <u>1</u>(1), 42-9 (1980).

7. Chau, Y. K., and M. E. Fox, "A GC Method for the Determination of Nitrilotriacetic Acid in Lake Water," _J. Chromatogr. Sci._, <u>9</u>(5), 271-5 (1971).

8. Aue, W. A., C. R. Hastings, K. O. Gerhardt, J. O. Pierce, II, H. H. Hill, and R. F. Moseman, "The Determination of Part-per-billion Levels of Citric and Nitrilotriacetic Acids in Tap Water and Sewage Effluents," _J. Chromatogr._, <u>72</u>(2), 259-67 (1972).

9. Malaiyandi, M., D. T. Williams and R. O'Grady, "A National Survey of Nitrilotriacetic Acid in Canadian Drinking Water," _Environ. Sci. Technol._, <u>13</u>(1), 59-62 (1979).

10. Williams, D. T., F. Benoit, K. Muzika, and R. O'Grady, "Gas Chromatographic Determination of Nitrilotriacetic Acid Using a Nitrogen Selective Detector," _J. Chromatogr._, <u>136</u>, 423-7 (1977).

11. Larson, R. A., J. C. Weston, and S. M. Howell, "Quantitative Gas Chromatographic Determination of Nitrilotriacetic Acid in the Presence of Other Carboxylic Acids," _J. Chromatogr._, <u>111</u>(1), 43-9 (1975).

12. Games, L., and J. A. Staubach, Procter and Gamble Co., "Analysis of Nitrilotriacetic Acid in Environmental Waters," submitted for publication to Environmental Science and Technology.

13. Warren, C. B., and E. J. Malec, "Quantitative Determination of Nitrilotriacetic Acid and Related Aminopolycarboxylic Acids in Inland Waters. Analysis by Gas Chromatography," _J. Chromatogr._, <u>64</u>(2), 219-37 (1972).

14. Stolzberg, R. J., and D. N. Hume, "Determination of Nitrilotriacetate in Environmental Water by Gas Chromatography of the Trimethylsilyl Ester," Anal. Chem., 49(3), 374-8 (1977).

15. Stolzberg, R. J., and D. N. Hume, "Determination of NTA in Environmental Water by Gas Chromatography," Anal. Lett., 6(9), 829-37 (1973).

16. Taylor, J. K., W. L. Zielinski, Jr., E. J. Maienthal, R. A. Durst, and R. W. Burke, "Development of Method for NTA (nitrilotriacetic acid) Analysis in Raw Water," Natl. Bur. Stand., Washington, D.C., U.S. Nat. Tech. Inform. Serv., PB Rep., 72 (No. 219035/3), 30 pp.

17. Rudling, L., "Determination of Nitrilotriacetic Acid," Water Res., 5(10), 831-8 (1971).

18. Longbottom, J. E., "Determination of Nitrilotriacetic Acid by High-Speed Ion-Exchange Chromatography," Anal. Chem., 44(2), 418-20 (1972).

19. Asplund, J., and E. Wanninen, "Polarographic Determination of Low Concentrations of Nitrilotriacetate in Lake Water," Anal. Lett., 4(5), 267-75 (1971).

20. Afghan, B. K., and P. D. Goulden, "Determination of Trace Quantities of NTA by Differential Cathode-Kay Polarography," Environ. Sci. and Technol., 5(7), 601-6 (1971).

21. Afghan, B. K., P. D. Goulden, and J. F. Ryan, "Automated Method for Determination of Nitrilotriacetic Acid in Natural Water, Detergents, and Sewage Samples," Anal. Chem., 44(2), 354-9 (1972).

22. Haberman, J. P., "Polarographic Determination of Traces of Nitrilotriacetate in Water Samples," Anal. Chem., 43(1), 63-7 (1971).

23. Hoover, T. B., "Polarographic Determination of NTA," Southeast Environ. Res. Lab., Athens, Ga., U.S. Environ. Prot. Agency, Off. Res. Dev., (Rep.) EPA, 73, (EPA-R2-73-254), 44 pp.

24. Stolzberg, R. J., "Determination of Ethylenediaminetetraacetate and Nitrilotriacetate in Phytoplankton Media by Differential Pulse Polarography," Anal. Chim. Acta, 92, 139-48 (1972).

25. Tabatabai, M. A., J. M. Bremner, and R. R. Storrier, "Extraction and Colorimetric Determination of Nitrilotriacetate (NTA) in Soils," Comm. in Soil Sci. and Plant Analysis, 3(1), 57-64 (1972).

26. Vanwelssenaers, N., and G. G. Clinckenmaillie, "The Determination of Nitrilotriacetic Acid in Granular Detergent Formulations with a Technicon AutoAnalyzer," Anal. Chim. Acta, 58, 243-5 (1972).

27. Swisher, R. D., M. M. Crutchfield, and D. W. Caldwell, "Biodegradation of Nitrilotriacetate in Activated Sludge," Environ. Sci. and Technol., 1(10), 820-7 (1967).

28. Horacek, J., and R. Pribil, "Potentiometric Determination of NTA, EDTA, and DTPA in Mixtures," _Talanta_, 16, 1495-9 (1969).

29. Sekerka, I., J. Lechner, and B. K. Afghan, "Potentiometric Determination of Nitrilotriacetate in Water and Sewage," _Anal. Lett._, 6(11), 977-83 (1973).

30. Lautenbacher, H. W., and H. W. Baker, "Determination of NTA in Aquatic Toxicity Studies: A Compleximetric Method Using Atomic Absorption Spectroscopy," _Bull. Environ. Contam. Toxicol._, 11(1), 57 (1974).

31. Davies, C., R. D. Hartley, and G. J. Lawson, "Chromatographic Behavior of Organic Acids on Dowex 1-X10," _J. Chromatogr._, 18, 47-63 (1965).

32. Daniel, R. L., and R. B. LeBlanc, "Polarographic Determination of Nitrilotriacetic Acid in (Ethylenedinitrilo)tetraacetic Acid," _Anal. Chem._, 31, 1221-3 (1959).

33. Woodiwiss, C. R., R. D. Walker, and F. A. Brownridge, "Concentration of Nitrilotriacetate and Certain Metals in Canadian Wastewaters and Streams: 1971-1975," _Water Res._, 13, 599-612 (1979).

34. Rudd, J. W. M., B. E. Townsend, and R. D. Hamilton, "Discharge of NTA From Two Sewage Treatment Facilities Located in a Midcontinental Climate," _J. Fish. Res. Board Can._, 30(7), 1026-30 (1973).

35. Rudd, J. W. M., and R. D. Hamilton, "Biodegradation of Trisodium Nitrilo-triacetate in a Model Aerated Sewage Lagoon," _J. Fish. Res. Board Can._, 29(6), 1203-8 (1972).

36. Shumate, K. S., J. E. Thompson, J. D. Brookhart, and C. L. Dean, "NTA Removal by Activated Sludge-Field Study," _J. Water Pollut. Contr. Fed._, 42(4), 631-40 (1970).

37. Renn, C. E., "Biodegradation of NTA Detergents n a Wastewater Treatment System," _J. Water Pollut. Control Fed._, 46(10), 2363-71 (1974).

38. Eden, G. E., G. E. Culley, and R. C. Rootham, "Effect of Temperature on the Removal of NTA (nitrilotriacetic acid) During Sewage Treatment," _Water Res._, 6, 877-83 (1972).

39. Bouveng, H. O., G. Davisson, and E. M. Steinberg, "NTA in Sewage Treatment," _Vatten_, 24, 348-359 (1968).

40. Colgate-Palmolive, unpublished data (1970).

41. Larson, R. J., G. G. Clinckermaillie, and L. Van Belle, "Effect of Temperature and Dissolved Oxygen on Biodegradation of Nitrilotriacetate," _Water Res._, 15(5), 615-20 (1981).

42. Epstein, S. S., "Toxicological and Environmental Implications of the Use of Nitrilotriacetic Acid as a Detergent Builder. 1," _Int. J. Environ. Stud._, 2(4), 291-300 (1972).