

challenge. Although there is no evidence that the effects observed in this experiment are species- or strain-dependent, data from a second species would aid in the interpretation of the results of this study and permit more precise estimates of the potential risks to humans from the consumption of nitrite. Such data could be developed only from other feeding studies requiring 3 to 4 years to complete. Since human exposure to nitrite is apparently greater than was previously recognized (20), a prudent course of action would include a gradual lessening of nitrite as a food additive as alternative plans are introduced to protect the public from botulism.

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6. Statistical testing was done by maximum likelihood fitting of all the data (except those for the positive controls) to the single-hit model. Two mathematical models were used. In the first model, probability of tumor development = $1.0 - \exp[\alpha - \beta(\text{dose})]$, where i indicates the experiment (the nitrite vehicle). For this model the β term was significantly different from zero ($P < .01$) and the overall fit gave a $G^2 = 10.94$ for 9 degrees of freedom ($P = .28$, indicating that the model cannot be rejected). Since the values of the constant α in the first model were similar, a second model was fitted: probability of tumor development = $1.0 - \exp[\alpha - \beta(\text{dose})]$. The parameters of this model were estimated to be $\alpha = -2.61 (\pm 0.13)$ and $\beta = 0.307 (\pm 0.107)$ (for dose in parts per thousand) and $G^2 = 14.76$ with 14 degrees of freedom ($P = .4$). The procedure followed is described in H. O. Hartley and R. L. Sielken, Jr. [*Biometrics* **33**, 1 (1977)].
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N-Formyliminodiacetic Acid, a New Compound from the Reaction of Nitrilotriacetic Acid and Chlorine

Abstract. It has been proposed that nitrilotriacetic acid be substituted for trisodium polyphosphates in detergents as a way to reduce the rate of eutrophication in the Great Lakes Basin. The reaction of nitrilotriacetic acid with chlorine-containing solutions produces a hitherto unknown degradation product, N-formyliminodiacetic acid, in high yield. The toxicological and environmental implications of this reaction are unclear.

In 1972 the Woods Committee, appointed by the U.S. Department of Health, Education, and Welfare to examine available evidence on the possible hazards of nitrilotriacetic acid [NTA or N,N-bis(carboxymethyl)-glycine] to human health, noted that there were "... no reports of mutational studies on possible NTA metabolites or on derivatives that might be formed in the environment prior to human exposure" (1, p. 21). One reaction on which information on derivatives was lacking was that of NTA and chlorine-containing solutions (2). This reaction might occur in the environment in any of several ways: (i) in wastewater treatment or water purification processes; (ii) in the wash cycle when NTA-containing detergents and commercial bleaches are mixed; or (iii) in the home when these products are mixed in concentrated form by the user or by children, either intentionally or accidentally. Morgenthaler and Langguth (3) studied the reaction of NTA and chlorine under simulated washing conditions and found that maximums of 10 percent NTA and 20 percent sodium hypochlorite (NaOCl) were lost; the reaction products, however, were not determined. Warren (4) studied the kinetics and mechanism of

NTA degradation at pH 5 to 7 and reactant concentrations comparable to those in the environment. Using derivatization and gas chromatographic-mass spectrometric techniques, he found that the principal products were iminodiacetic acid, glyoxylic acid, and glycine when degradation of the NTA was incomplete.

Our studies of this reaction developed out of our interest in learning whether the same products were formed at pH 11 as at pH 5. By using high reactant concentrations and ^{13}C nuclear magnetic resonance (NMR) spectroscopy, we were able to examine the solutions in situ, thereby minimizing the risks of destroying more labile intermediates or creating artifacts through manipulation of the solutions.

When 1 g of the trisodium salt of the monohydrate of NTA (Monsanto Industrial, St. Louis) was mixed at room temperature with 15.0 ml of NaOCl solution ($[\text{NaOCl}] = [\text{NaCl}] = 0.7M$, pH 11.0), the solution became exothermic (55° to 60°C) after 30 to 45 seconds, fine bubbles evolved, and the pH of the solution dropped to 8.0. The presence of N_2 and CO_2 and the absence of volatile chloramines and NH_3 in the evolved gases were confirmed in separate experiments that allowed porting of the gases directly into a mass spectrometer.

After the bubbling had stopped (< 5 minutes), we examined the solution by ^{13}C NMR spectroscopy. Several new signals (Fig. 1) were observed. Singlets at 171.9, 162.0, and 83.0 parts per million (ppm) (5) were identified as formate (HCOO^- , a minor component), bicarbonate (HCO_3^-), and formaldehyde (as trioxymethylene), respectively, from spectra of aqueous solutions of these species at pH 8.0. The NTA signals were unchanged at 176.8 (carboxyl) and 59.5 (methylene) ppm but had diminished in intensity by 85 percent.

Five signals remained unidentified. The offspin resonance coupling of these signals showed two singlets at 177.1 and 176.1 ppm, a doublet at 166.9 ppm, and two triplets at 53.1 and 48.3 ppm. The relative intensity of these signals remained constant from experiment to ex-

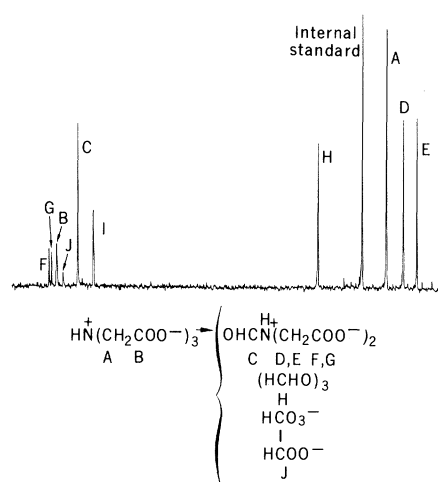
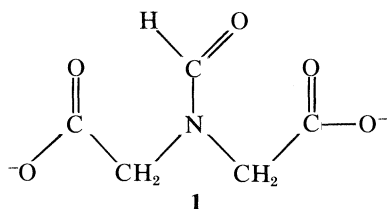


Fig. 1. The ^{13}C NMR spectrum of 1 g of the trisodium salt of the monohydrate of NTA in 15 ml of NaOCl (0.7M) solution after 10 minutes of reaction time. The internal standard was dioxane (Varian XL-100 NMR spectrometer).

periment. This constancy, plus the resemblance of the proton NMR spectrum of the reaction products to that of dimethylformamide, suggested that the five unknown signals might be associated with a single species, possibly the *syn* and *anti* forms of the *N*-formyl derivative of iminodiacetic acid (IDA), a new compound (1) not previously reported in the literature:



In an effort to demonstrate whether this was the case, we prepared an authentic sample of *N*-formyliminodiacetic acid {FIDA or [*N*-(formyl)-*N*-(carboxymethyl)-glycine]} by an alternate route. We refluxed the disodium salt of IDA (Aldrich Chemical; 20g, containing 2 percent by weight of NTA as an impurity) with 100 ml of 90 percent HCOOH at 150°C for 10 minutes (> 95 percent conversion) (6). The solution was distilled under a vacuum overnight to remove the bulk of the HCOOH, and the residual solution was brought to pH 9 with 10 percent NaOH. The synthesized compound was positively identified as FIDA on the basis of elemental and spectral analysis (7). The ¹³C NMR spectrum of the synthesized FIDA was coincident with that of the five unknown signals in the solution containing the products of the NTA-chlorine reaction.

The product yields from the NTA-chlorine reaction at pH 11.0 were estimated from the NMR peak heights to be as follows: FIDA, 1.46 mmole (40 percent yield); trioxymethylene, 0.36 mmole; HCO₃⁻, 0.73 mmole; and HCOO⁻, 0.13 mmole. The amount of unreacted NTA was 0.52 mmole or 15 percent of the starting material. We have also found that FIDA is produced at pH 5.0 from NTA and hypochlorous acid under otherwise identical conditions to those at pH 11.0, although apparently in somewhat lower yields (~ 10 percent). Formaldehyde (trioxymethylene) was also present, and so was IDA when the reaction solution contained phosphate buffer for pH stabilization (8).

To provide a preliminary assessment of the potential hazard of FIDA, we evaluated the synthesized material in the Ames assay using five strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538) with and without metabolic activation (9). We

tested the FIDA twice in water at six dosages ranging from 10 to 5000 μg per plate. No mutagenic response was observed at any dosage, an indication that FIDA is not a potent mutagen.

This report is a further step toward addressing the concerns originally voiced by the Woods Committee over a need for information on NTA derivatives that might be formed from reactions in the environment and on their mutagenic potential. The lack of mutagenicity of FIDA in the Ames test is reassuring but, because this compound is new, other toxicological and environmental data should be sought as part of the continuing program to ensure the safety of NTA input into widespread use (10). Research on the reactivity of NTA with other oxidants in the environment is also warranted for the same reason.

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7. The proton spectrum showed two methylene singlets at 84.21 and 4.29 and a formyl singlet at 88.32 corresponding to the monosodium salt of *N*-formyliminodiacetic acid in D₂O (84.89). The infrared spectrum showed the following absorbances (KBr pellet): 3400 (OH), 2950 (C-H), 2380 (N⁺-H), 1660 (C=O), 1450, 1430, 1380, 1300, 1000, 875, 750, and 705 cm⁻¹. The ultraviolet spectrum (H₂O): maximum wavelength λ_{max} = 196 nm, molar absorptivity = 6200. Elemental data were consistent for a mixture containing 80 percent of the monosodium salt hydrate and 20 percent of the disodium salt hydrate. Calculated percentages: C, 29.26; H, 3.58; N, 6.82; Na, 13.27; values found: C, 29.67; H, 3.75; N, 6.74; Na, 13.49.
8. Not all signals were identified at pH 5.0, and minor amounts of other products may be formed at both experimental pH values studied (pH 5 and 11). A fragment, identified as diformylglycine [*N,N*-bis(formyl)-glycine], was observed in the gas chromatographic-mass spectrometric spectrum of the reaction solution, but neither this compound nor the FIDA was quantifiable because of the lability of the formyl group in strong acid during derivatization. When NTA, IDA, or glycine (2.3 × 10⁻³M) and excess NaOCl (0.02 to 0.07M) were reacted at 55°C and pH 10, the ratios of OCl⁻ consumed to the initial amine concentration were 10.5, 7.5, and 4.5, respectively, signifying complete decomposition to CO₂, N₂, H₂O, and Cl⁻. The rate of bleach loss, monitored iodometrically, was at least five times as fast with IDA and glycine as with NTA.
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10. Great Lakes Research Advisory Board, Annual Reports to the International Joint Commission, Windsor, Ontario, July 1977 and 1978. After reviewing all of the then available literature and Canadian experience, the board found (pp. 26 and 27 of the 1978 report) "no reasonable cause for restricting the use of NTA as a replacement for phosphate in laundry detergents in the Great Lakes Basin." The reports concluded that NTA was environmentally safe to use as a means of reducing phosphorus inputs into the basin in order to slow the rate of eutrophication in those waters, a process of serious concern to both Canadian and U.S. governments. In coming to this conclusion, the board accepted the recommendations of its task force on ecological effects to conduct certain additional studies and monitoring programs on NTA in the environment.

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DNA Organization of *Methanobacterium thermoautotrophicum*

Abstract. *The organism Methanobacterium thermoautotrophicum, an archaeobacterium, is evolutionarily very distant from both traditional prokaryotes and eukaryotes. Its genome (DNA) has physical characteristics typical of most prokaryotes except that it is quite small (about 10⁹ daltons, less than half the size of the genome of Escherichia coli) and contains a significant amount (6 percent) of DNA which renatures extremely rapidly.*

Studies by Woese and Fox (1) indicate that the traditional classification of life into two kingdoms, prokaryotes and eukaryotes, should be replaced by three categories or lines of descent, which Woese and Fox call archaebacteria, eubacteria, and urkaryotes. They propose these divisions because the comparison of two-dimensional nucleotide patterns (fingerprints) of 16S and 18S RNA's indicates that these three groups are evolutionarily equidistant from each other.

The eubacteria and urkaryotes correspond roughly to the conventional categories of prokaryotes and eukaryotes, respectively. For example, the eubacteria comprise the typical bacteria (such as *Escherichia*, *Eubacterium*, *Micrococcus*, and *Spirochaeta*) as well as blue-green algae and chloroplasts. The urkaryotes comprise the typical eukaryotes such as animals, plants, fungi, and slime molds; or, more precisely, urkaryotes could be said to comprise the