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Nitrification of Triazine Nitrogen

Nitrification of N released from cyanuric acid, ammelide, ammeline, or melamine varied inversely with the number of amino groups on the triazine ring. Nitrification of melamine and cyanuric acid powders was slightly greater than solutions and considerably greater than -8+12 mesh granules of these materials. Melamine and cyanuric acid solutions perfusing through soil slightly inhibited the rate of nitrate formation from added ammonium, and caused a marked but temporary accumulation of nitrite. More nitrate was produced from triazine incubated in soil bottle cultures than from triazine perfusing through soil.

UREA heated under NH_3 pressure to temperatures of $150^\circ C.$ and above yields a six-membered ring containing three nitrogen and three carbon atoms. Hydroxyl groups attached to the ring carbon may be partially or completely replaced by amino groups as temperature and pressure are increased, resulting in the series of compounds—cyanuric acid, ammelide, ammeline, and melamine—with none, one, two, or all hydroxyls replaced, respectively. The structural formulas of these compounds are given by Terman *et al.* (10).

Scholl *et al.* (8) found that about 1% of the nitrogen in the melamine portion of melamine nitrate, phosphate, or sulfate nitrified during 13 weeks' incubation in Norfolk loamy fine sand. Clark *et al.* (2) reported that potassium and sodium cyanurates nitrified slowly for 6 weeks, followed by a more rapid and complete release of organic nitrogen between 6 and 9 weeks. Ammeline and mixtures of ammelide and ammeline nitrified at maximum rates between the 9- and 12-week incubation periods. Very little nitrification of melamine was observed over a 15-week period. Konishi and Imanishi (6) concluded that ammeline and melamine ammonified very slowly during 10 weeks in a paddy soil.

The chemical and biochemical factors which lead to degradation of the triazines are of agronomic interest with reference to both their possible use as nitrogen fertilizers and the actual use of substituted triazines as herbicides. Slight modifications of the basic triazine structure have been shown (5) to result in marked changes in biological activity. On the other hand, a possible increase in rate of chemical hydrolysis may be achieved through partial acidulation, or through addition of heavy metal ions to the triazine. This paper will

Table I. Summary of Test Materials

Material	N, %	Source
Urea	46.1	Grace Chemical Co.
Cyanuric acid	32.4	Eastman Organic Chemicals
Ammelide	43.7	American Cyanamid Co.
Ammeline	49.4	American Cyanamid Co.
Melamine	66.5	American Cyanamid Co.
Melamine phosphate (MP)	39.1	TVA
Melamine nitrate (MN)	51.4 (3.9% $NO_3^- N$)	TVA
Melamine phosphoric acid (MPA)	54.3	TVA
Melamine nitric acid (MNA)	60.3 (2.6% $NO_3^- N$)	TVA
Melamine ferric ammonium sulfate (MFe)	55.5 (0.4% $NH_4^+ N$)	TVA
Melamine copper sulfate (MCu)	60.2	TVA
Melamine cadmium sulfate (MCd)	62.0	TVA
Urea-formaldehyde (activity index 51.7)	38.6	Borden Co., Chemical Division

report further nitrification tests made on some modified triazine compounds to study their degradation in soil.

Materials and Methods

Nitrogen Sources Studied. Reagent grade cyanuric acid, ammelide, ammeline, and melamine were obtained from chemical supply companies. These materials were further processed by personnel of the Applied Research Branch, Division of Chemical Development, TVA, to form compounds or mixtures (Table I) as follows.

MELAMINE PHOSPHATE (MP) AND MELAMINE NITRATE (MN). Twenty grams of melamine and 25 grams of 85% phosphoric acid or 25 grams of 65% nitric acid were allowed to react in 500 ml. of boiling water. After cooling, the precipitate of melamine phosphate or nitrate was collected on a filter, recrystallized from 400 ml. of boiling water, washed, and then dried at $150^\circ F.$

MELAMINE ACID AND METAL ION, GRANULES. The indicated parts by

weight of melamine and acid or salt were mixed with water to form a paste, dried at $150^\circ F.$, crushed and screened.

(MPA) Melamine phosphoric acid granules: 10 parts melamine to 2.29 parts 85% phosphoric acid.

(MNA) Melamine nitric acid granules: 10 parts melamine to 1.92 parts 65% nitric acid.

(MFe) Melamine ferric ammonium sulfate granules: 10 parts melamine to 1.72 parts $Fe_2(SO_4)_3 \cdot (NH_4)_2SO_4 \cdot 24H_2O$.

(MCu) Melamine copper sulfate granules: 10 parts melamine to 0.79 part $CuSO_4 \cdot 5H_2O$.

(MCd) Melamine cadmium sulfate granules: 10 parts melamine to 0.46 part $CdSO_4 \cdot 8H_2O$.

Soils. Two soils were used in the experiments—Webster silty clay loam (Iowa), pH 8.2; Hartsells fine sandy loam (Alabama), pH 5.2.

Incubation Experiments. The materials under test were incubated with soil contained in 125-ml., square milk-dilution bottles, 500-ml. Erlenmeyer flasks, or waxed cartons. Soil mois-

Table II. Nitrification of Urea, Urea-Formaldehyde, and Some Triazine Derivatives in 100 Grams of Webster Silty Clay Loam

N Source ^a	% of Organic N ^b Found as NO ₃ ⁻ N in Weeks:	
	10	28
Control
Urea prills	79.7	82.7
Ureaform	22.8	25.5
MP	1.3	0.2
MN	0.3	4.6
MFe	1.1	0.1
MCu	1.0	None
MCd	0.4	None
MPA	1.0	None
MNA	None	None
Melamine	0.7	None
Ammeline	0.6	5.7
Ammelide	2.9	16.8
Cyanuric acid	34.8	72.9

^a All materials were added to duplicate soil samples as granules or prills screened to pass -8+12 mesh.
^b Total NO₃⁻ N found corrected for control and NH₄⁺ and NO₂⁻ N incubated with triazine.

Table III. Nitrification of Triazine N in Two Soils

N Source ^a	Form	% Triazine N Nitrified in Weeks:			
		6	12	18	24
WEBSTER SILTY CLAY LOAM					
Cyanuric acid	Solution	6.9	69.2	83.9	86.4
	Powder	6.9	66.4	88.9	92.2
Ammelide	Powder	21.7	29.1	78.7	78.3
Ammeline	Powder	16.6	30.7	51.7	69.4
Melamine	Solution	6.4	9.1	10.5	13.7
	Powder	7.7	9.1	15.4	17.9
Urea	Prill (-8+12 mesh)	88.2	93.0
Ureaform	-8+12 mesh	14.0	26.2
HARTSELLS FINE SANDY LOAM					
Cyanuric acid	Powder	15.0	64.1	55.0	69.7
Ammelide	Powder	11.0	79.0	89.4	78.1
Ammeline	Powder	6.9	4.0	10.4	31.1
Melamine	Powder	0	0	3.9	8.9
Urea	Prill (-8+12 mesh)	53.1	80.6
Ureaform	-8+12 mesh	0	8.6

^a In each sample, 7.0 mg. of triazine N was added to 30.0 grams of soil.

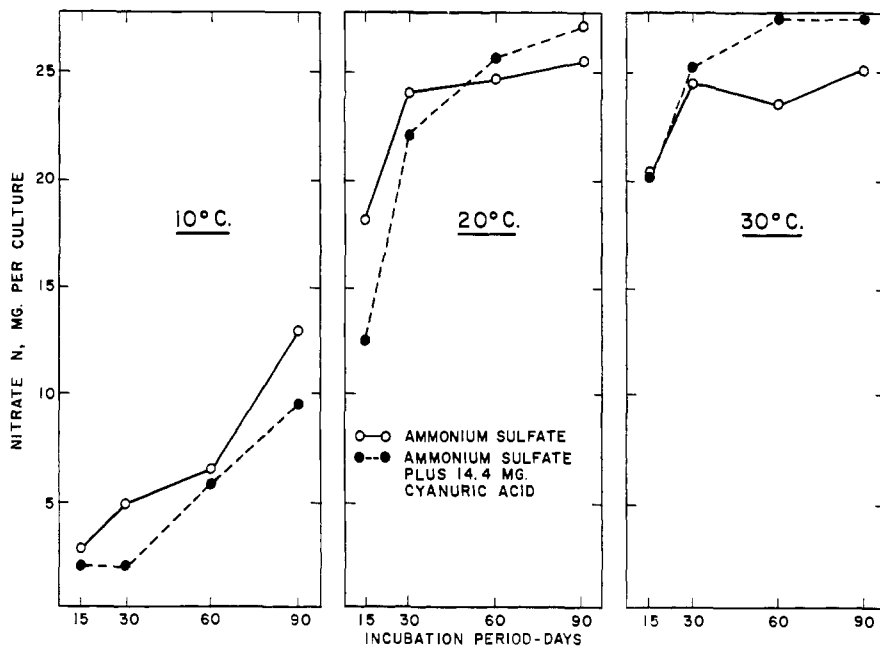


Figure 1. Effect of cyanuric acid on the nitrification of 20 mg. of N as (NH₄)₂SO₄ at three temperatures

ture was brought to 60% of field capacity and maintained at that level. A 1-hole rubber stopper allowed for moderate aeration of the culture while minimizing moisture loss. Fresh air was drawn through each flask at weekly intervals. All bottle cultures were incubated in a moist atmosphere at 32° C. for periods up to 28 weeks.

In experiment 1, duplicate samples of about 200 mg. of test material were mixed with 100 grams of Webster soil in Erlenmeyer flasks. In experiment 2, 7.0 mg. of triazine or urea nitrogen were added either as solid or in solution to

30 grams of Webster soil and similar amounts of solid materials were added to Hartsells soil in milk-dilution bottles. In a third experiment, 14.4 mg. of cyanuric acid and 20.0 mg. of nitrogen as ammonium sulfate were applied in a single spot 0.5 inch below the surface of 200 grams of Hartsells soil in waxed cartons and incubated at either 10°, 20°, or 30° C. for periods up to 90 days.

Perfusion Experiments. Nitrification of the triazines and their effects on the oxidation of added ammonium were studied by the perfusion technique. Dilute solutions of triazines were con-

tinuously circulated in a well aerated closed system, the triazine solutions passing through 30 grams of Krillium-treated Webster soil at 25° C. for periods up to 165 days. Treatment consisted of mixing a 0.1% aqueous solution of Krillium with soil to form a thick slurry, followed by drying and granulation. Samples of pot solution were taken at weekly intervals for nitrite and nitrate analysis.

Results

Incubation Experiments. Nitrification of the -8+12 mesh test materials in Webster soil (experiment 1) over a 28-week period is summarized in Table II. Duplicate samples were highly reproducible, with the exception of those receiving ammelide. About 80 and 23%, respectively, of the added urea and ureaform nitrified within 10 weeks and little additional nitrate nitrogen over control was found upon further incubation. About 1% of the nitrogen from melamine, regardless of whether the melamine had been further processed, was found as nitrate after 10 weeks. Nitrate levels in these samples dropped, sometimes slightly below the controls, over the 28-week incubation period, with the exception of nitrate from melamine nitrate. This apparent loss of nitrate nitrogen can possibly be explained by increased nitrate assimilation by the soil micropopulation in the treated soils. Nitrate recovered from melamine nitric acid, when corrected for nitrate contained in the triazine, was lower than control for both 10- and 28-week incubation periods.

Melamine phosphate, in agreement

with the work of Clark *et al.* (2), was found to nitrify at a slightly faster rate than melamine, but the differences were small. Almost 5% of the nitrogen from the melamine portion of melamine nitrate was recovered as nitrate after 28 weeks. Except for the slight increase in nitrification noted for melamine nitrate, stimulation of biochemical degradation of melamine with added nitrogen, phosphorus, or iron, or catalysis of chemical hydrolysis with copper and cadmium was not observed in any of the samples. The biochemical degradation of the triazines, as reflected by the nitrification data, decreased as the number of amino groups on the triazine ring increased.

In experiment 2, the nitrification of triazine nitrogen added as a powder was followed over a 24-week period in both Hartsells and Webster soils. In addition, a comparison of nitrification rates of melamine and cyanuric acid added as either powder or solution was made in Webster soil. As in experiment 1, duplicate samples were highly consistent with the exception of ammeline and cyanuric acid nitrifying in the Hartsells soil.

As shown in Table III, the extent of nitrate formation in both soils varied inversely with the degree of amination of cyanuric acid. A few inconsistencies were observed for the ammeline and cyanuric acid treatments after 12 to 18 weeks' incubation in Hartsells soil. Nitrate values for these treatments fluctuated widely between replicates. The reason is not apparent.

Powdered melamine and cyanuric acid nitrified to a slightly greater degree than solutions of these materials, while both powders and solutions of the two triazines nitrified to a much greater extent than the -8 + 12 mesh granules used in experiment 1 in the same soil and under similar conditions.

In another test, three rates of nitrogen as either melamine or cyanuric acid were incubated in 30.0 grams of Webster soil for 24 hours. The per cent of the total triazine nitrogen which was recovered as nitrate decreased with increasing rates of application, being 17.9, 7.0, and 4.2% of the 7, 14, and 21 mg. rates of nitrogen as melamine, respectively, and 92.2, 91.5, and 85.5% of the same rates, respectively, as cyanuric acid.

A third experiment was conducted to determine the effect of cyanuric acid on nitrification of added ammonium, as affected by incubation period and temperature. Nitrification was inhibited by cyanuric acid at 10° C. for 90 days, and for about 30 days at 20° or 30° C., after which time degradation of triazine was observed. After 90 days' incubation at 20° and 30° C., 12.5 and 15.3% of triazine nitrogen was apparently recovered as nitrate, assuming 100% oxidation of the added ammonium.

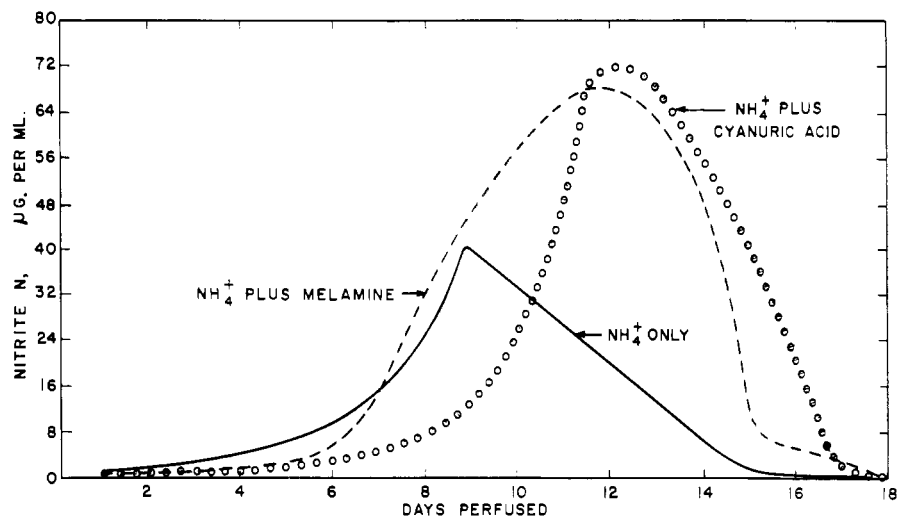


Figure 2. Oxidation of ammonium to nitrite, as affected by cyanuric acid and melamine

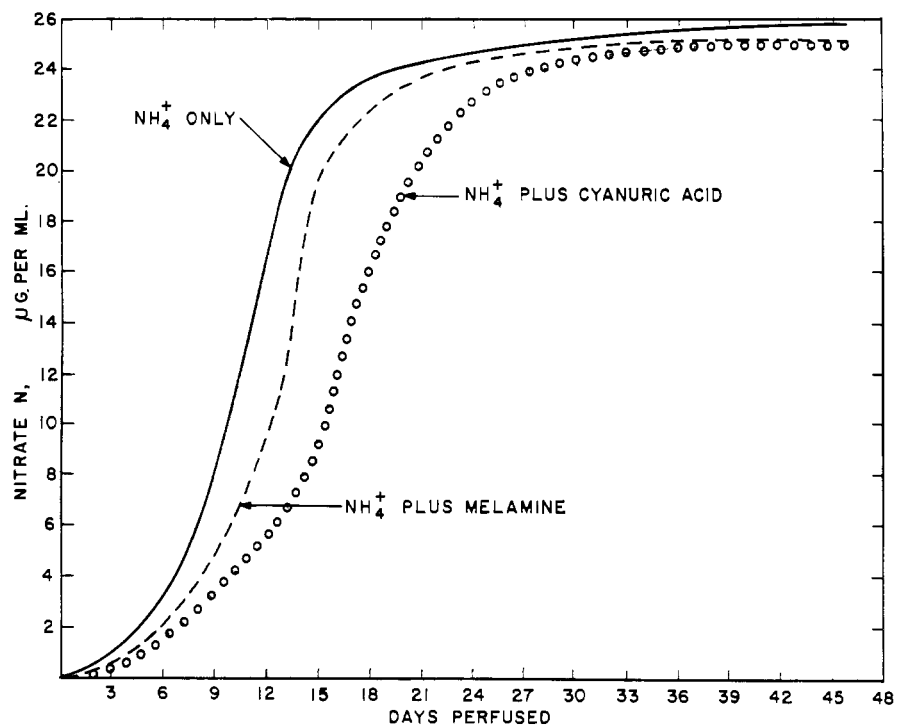


Figure 3. Oxidation of ammonium to nitrate, as affected by cyanuric acid and melamine

The relationships are shown in Figure 1.

Perfusion Experiments. The materials under test were perfused in solution through Webster soil. In the first run, the nitrification of 2 to 5 mg. of nitrogen as triazine or as a purified urea pyrolyzate mixture (49.1% N) was followed over a 6-week period. No nitrite or nitrate nitrogen above control was formed during this time. Nitrate formation from mineralized soil nitrogen was inhibited by the presence of triazine, the amount of inhibition varying inversely with the degree of amination of the triazine ring.

In the second run, 10 mg. of nitrogen as either melamine or cyanuric acid

alone, or supplemented with 10 mg. of ammonium nitrogen was perfused through Webster soil for 165 days. Additional 2- or 4-mg. amounts of ammonium nitrogen were added after 60, 116, and 144 days to the cultures which had originally received ammonium. Nitrite formation for the first 18 days and nitrate formation over 48 days are indicated by Figures 2 and 3.

Maximum nitrite formation from ammonium N in the absence of triazine occurred during the period 7 to 9 days after starting the perfusion of the dry soil. Nitrite levels were negligible after 15 days' perfusion. Nitrate formation increased rapidly during the period 10 to

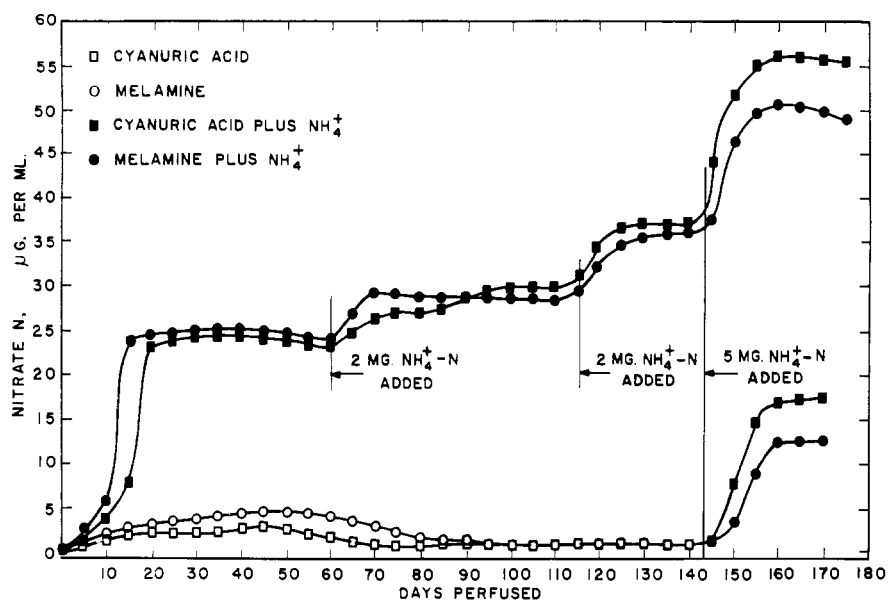


Figure 4. Oxidation of successive additions of ammonium, as affected by cyanuric acid and melamine

14 days from zero time. However, in the presence of either melamine or cyanuric acid, an approximate 2-day lag occurred in the oxidation of the supplemental ammonium N to nitrite, with a corresponding 2- and 3-day lag in nitrate formation from ammonium originally added with melamine and cyanuric acid, respectively. Nitrite accumulated to a greater degree in perfusates that originally contained ammonium and triazine N than ammonium alone, maximum average contents being 39.9, 68.9, and 72.0 µg. of nitrite nitrogen per ml. for the ammonium, melamine-ammonium, and cyanuric acid-ammonium systems, respectively. Cyanuric acid caused a greater lag than melamine in both nitrite and nitrate formation from added ammonium. After 18 days' perfusion, nitrite levels in the triazine-ammonium systems were negligible (compared to 15 days for ammonium perfusing alone), and oxidation of ammonium to nitrate in the presence of either triazine was 99% complete within 40 days.

Melamine and cyanuric acid perfusing without added ammonium (other than that derived from soil organic matter) degraded at a slow but constant rate for about 4 weeks, after which time nitrate assimilation in the perfusion system occurred faster than nitrate production. After 46 days, a maximum of 14.0 and 9.8% of nitrogen apparently originating from melamine and cyanuric acid, respectively, was found as nitrate.

Ammonium nitrogen added after 60, 116, and 144 days was completely nitrified within 7 days of addition in the ammonium alone or melamine-ammonium systems. However, in the cyanuric acid system, a lag period of almost 5 weeks occurred before the

ammonium N added after 60 days was completely oxidized. The lag period was reduced to about 2 weeks for the subsequent 116- and 114-day additions, and a small amount of nitrate nitrogen above that expected from the added ammonium nitrogen was formed.

These relationships are presented in Figure 4. In additional experiments with triazine-urea and triazine-urethane systems (not recorded here), similar inhibition of the oxidation of nontriazine N for comparable periods of time have been observed.

Discussion

The complete chemical degradation of melamine to ammonium by strong acids or the partial alkaline hydrolysis of the amino groups of melamine present little difficulty in the laboratory. However, the biochemical degradation of melamine proceeds at a very slow rate in soils. Perhaps this is due in part to the symmetrical resonating structure of the molecule. Polymerization resulting in an increase in symmetry leads to stability, while the labile products of biochemical transformations tend to asymmetry.

In general, the asymmetrically-substituted triazines—e.g., atrazine—now finding use as weed killers, are reported by Gysin (5) to be much more rapid in herbicidal activity than symmetrically-substituted molecules of similar composition, such as simazine or propazine. Current work shows that chlorotriazines with asymmetrical substitution on the number 4 and number 6 carbons are degraded to yield nitrate at a slightly faster rate than the corresponding symmetrically-substituted chlorotriazines.

An increase in number of basic groups

and molecular charge due to amination of cyanuric acid to the triamine (melamine) may increase the tendency of the molecule to react with acidic groups of proteins or nucleic acids, thereby interfering with soil micro-organism enzyme systems. The effect on physiological activity through the introduction of amino groups is well known (9). Introduction of hydroxyl groups markedly affects the physicochemical properties of organic substances, usually leading to better orientation of the molecule at lipid-water interfaces.

A temporary lag in nitrification was observed in the systems where ammonium was perfusing in the presence of triazine, indicating a competitive inhibition of enzyme function that is short-lived, due either to adaptation of the micro-organisms to the inhibitory principle, or to masking of the inhibition as the numbers of nitrifying bacteria increased. *Nitrobacter* was affected to a greater degree than *Nitrosomonas*, as evidenced by nitrite accumulation in the ammonium-triazine systems. Cyanuric acid, ammeline, or melamine, per se, did not kill the nitrifying organisms since supplemental ammonium was completely oxidized in their presence, after the initial lag period. The increase in rate of oxidation of the supplemental ammonium after the lag period, however, was not preceded by increased destruction of triazine.

In bottle cultures, the rate of triazine breakdown decreased with increasing amounts applied to soil, indicating increasing inhibition of the enzyme systems involved in the initial breakdown of triazine, leading to ammonification.

The observed differences in biochemical behavior of cyanuric acid and melamine cannot be explained by differences in solubility. In the perfusion experiments, all triazines were added in solution, and in the bottle cultures, either solutions or solid particles of cyanuric acid degraded to a greater extent than the corresponding forms of melamine. However, powdered melamine or cyanuric acid nitrified at a faster rate than either -8+12 mesh particles or solutions. Tribe (11) reported that fungal decomposition of cellulose was slightly more rapid from 0.5- × 1.0-cm. pieces of cellulose film than from powder, probably because the larger pieces offered a greater continuous surface area for the extension of fungal mycelium. As the cellulose was lysed, the resulting liquid film was quickly colonized by bacteria and protozoa. Guillemat (4) identified some soil fungi capable of degrading simazine and concluded that this triazine does not affect the balance of fungi or bacteria in soil. Reid (7) reported at least one species of bacteria belonging to the *corynebacteriaceae* which can attack triazine structures.

An initial fungal attack on the triazine ring, followed by ammonification and nitrification, may explain why the powdered material degraded at a faster rate than its solution, while the relatively fewer particles in the -8+12 mesh treatments provided considerably less surface area and were thus degraded at the slowest rate. In addition, less nitrogen was released from cyanuric acid perfusing through soil, in a system more conducive to bacterial activity, than from cyanuric acid incubated in the same soil contained in a bottle. Neither metal ion catalysis of triazine hydrolysis in soil nor stimulation of biochemical degradation was observed in experiment 2. Of interest in this connection, however, is the report of Castelfranco and Deutsch (7) on the acceleration of simazine breakdown by polysulfides. Cox (3) has also noted that methylmercapto-triazines have quick action and short persistence in soils. Current work in the authors' laboratory indicates that methoxy- or thiomethoxy-substituted triazines degrade faster than the corresponding chlorotriazines.

Although the few cropping experiments with compounds of the cyanuric

acid series (10) have not shown these materials to advantage, laboratory studies, coupled with their relative ease of production, high N content, and possibility of slow N release in soil, suggest that they are compounds worthy of further study as possible nitrogen fertilizer materials.

Slight modifications of structure may result in materials less resistant to degradation and more amenable to eventual plant uptake of contained N. The dependence of cyanuric acid breakdown on temperature may offer a possible use of this material as a fall-applied nitrogen source supplemented with a readily available source in the spring.

Since materials such as cyanuric acid have been shown to be temporarily toxic to seedlings (10) as well as to inhibit microorganism activity, placing the materials near the seed should be avoided. Further, evaluation of the material as a slow-release nitrogen source should be made only on crops that are expected to respond to smaller amounts of nitrogen added at frequent intervals during the season.

Acknowledgment

Credit is due J. D. DeMent and C. M. Hunt for the data listed under Incubation Experiment 3 and Figure 1.

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FERTILIZER NITROGEN SOURCES

Crop Response to Urea and Urea Pyrolysis Products

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Availability of nitrogen in urea and urea pyrolysis products for four crops of corn forage and for four clippings of Coastal Bermudagrass grown in greenhouse cultures decreased as follows: urea > cyanuric acid > ammelide > urea pyrolyzate > ammeline > melamine. Cyanuric acid was toxic initially, but most of its nitrogen became available for succeeding crops. Urea formaldehyde was similar in effect to ammeline and urea pyrolyzate for Bermudagrass. In field experiments with wheat, urea pyrolyzate and urea formaldehyde were 33 and 49%, respectively, as effective as urea, and the other sources were even less satisfactory.

PYROLYSIS of urea at moderately high temperature under pressure results in the formation of mixtures of water-insoluble compounds which may have value as nitrogen fertilizers. These compounds are heterocyclic C—N materials and vary in the degree of substitution of amine for hydroxyl groups. The structural formulas of the series (triazines) are shown in Figure 1. Substitution of an NH₂ group for one OH

group in cyanuric acid yields ammelide, substitution of two OH groups yields ammeline, and substitution of all three OH groups with NH₂ yields melamine. Generally, chemical activity of the compounds decreases with increasing substitution. In addition to these compounds, pyrolyzed urea also contains unreacted urea and may contain other compounds as well. Substitution of various organic groups yields other triazine compounds, many of which have been widely used as herbicides.

Kazarnovskii and Malkina (2) studied the kinetics of formation of intermediate

reaction products and melamine. They showed that heat treatment of urea resulted in the formation of ammonium cyanate and its decomposition into cyanic acid and ammonia. Cyanuric acid is formed by the polymerization of cyanic acid. Ammelide, ammeline, and melamine are amination products of cyanuric acid. Kinoshita (3, 4) previously reported on the synthesis of melamine from urea.

Scholl *et al.* (5) found in greenhouse pot tests that response of millet to the nitrogen in melamine nitrate was nearly as great as that in urea and sodium

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