

## Thiourea Solutions as Nitrification Inhibitors

John T. Hays\* and Donald J. Forbes

Thiourea inhibits the growth of *Nitrosomonas* and prevents active nitrification until its concentration has been reduced to a minimum level by soil organisms. The solubility of thiourea in water is about 12%, but it is much more soluble in ammonia solutions. A solution containing 28% thiourea, 20% ammonia, and 50% water has been prepared, as well as solutions of thiourea with ammonia, urea, and ammonium nitrate, containing 9–21% thiourea. Salting out temperatures are

given for these solutions. Incubation studies of nitrification indicate that a concentration of 4–8 mg of thiourea nitrogen per 100 g of soil inhibits nitrification for 3–6 weeks. Low cost, large volume production of thiourea and demonstration of effectiveness in the field at concentrations in the lower portion of the active range are required to make thiourea competitive with more active nitrification inhibitors.

The nitrification inhibitor approach to controlled release of nitrogen fertilizers has the attractive feature of supplying nitrate at a rate approximating plant growth curves. The initial delay phase caused by inhibition of *Nitrosomonas*, corresponding to the initial low demand for nitrogen in the early stages of growth, is followed by uninhibited nitrification, corresponding to a rapid rate of growth. Thus, ammonium nitrogen, which is held relatively immobile in most soils, is not converted to mobile nitrate until the growing plant is able to utilize increased amounts of nitrate and to prevent its movement out of the root zone.

Many compounds have been shown to act as nitrification inhibitors; a number are undergoing commercial development, particularly in Japan (Hauck and Koshino, 1971; Hauck, 1972). Thiourea is a recognized member of this group and has been carefully investigated by McBeath (1962) and also by Fuller and coworkers (Fuller *et al.*, 1950; Fuller, 1963). In this paper we report effects of thiourea on nitrification in incubation experiments. We have also prepared fertilizer solutions containing relatively large amounts of thiourea and have demonstrated the inhibiting effects of such solutions on nitrification.

### EXPERIMENTAL SECTION

**Reagents** used were: ammonium hydroxide, concentrated, reagent grade, 28%  $\text{NH}_3$  (Mallinckrodt); ammonium nitrate, reagent grade (Mallinckrodt); thiourea, technical grade (Millmaster International); urea, crystalline (Mallinckrodt).

**Procedures.** *Nitrification.* The nitrification experiments were carried out as previously described (Clark *et al.*, 1959; Hays *et al.*, 1965) for the solids used. The solutions were used directly and also in amounts to provide 20 mg of nitrogen per flask containing 100 g of soil. Due to the inhibition of nitrification of soil nitrogen, the total amount of nitrate nitrogen measured is reported (Table I). In Table III, the per cent conversion is reported to put results on a comparable basis.

*Preparation of Solutions.* The reagents were added to distilled water or to concentrated ammonium hydroxide. Thiourea was generally added last. Solutions were mechanically stirred and, where necessary, were warmed to 30–40° to effect solution.

*Salting Out Temperatures.* No carefully standardized procedure was used. Solutions were allowed to stand at room temperature; if no solids separated, solutions were cooled in an ice bath while being stirred with a thermometer to get an estimate of the salting out temperature.

*Analyses.* The values for total nitrogen in Table II are analytical values determined for the nitrification procedure.

### RESULTS AND DISCUSSION

**Perfusion and Incubation Experiments.** McBeath (1962) has established that thiourea inhibits the growth of *Nitrosomonas* and that it is decomposed by soil organisms. Active nitrification does not occur until the concentration of thiourea has been reduced below a minimum level. In soil perfusion experiments McBeath (1962) showed a significant effect on nitrification at a thiourea concentration of  $3.3 \times 10^{-4} M$  in the perfusion liquid, with a more pronounced inhibition at  $10^{-3} M$ . These concentrations amount, respectively, to 9 and 28 mg of thiourea per 100 g of soil (400 ml of perfusion liquid per 100 g of soil). In parallel experiments with 2-chloro-6-(trichloromethyl)pyridine (Dowco 163 or N-Serve, Dow Chemical Co.), a concentration of 10  $\mu\text{g}$  of this material/g of soil or 1 mg/100 g of soil gave inhibition comparable to but somewhat more pronounced than 9 mg ( $3.3 \times 10^{-4} M$ ) of thiourea. Thus, about ten times as much thiourea as 2-chloro-6-(trichloromethyl)pyridine is required in laboratory experiments to give inhibition of nitrification for periods of 3–6 weeks. This concentration disadvantage makes the quantities required in the field and the mode of application important in the practical consideration of thiourea as a nitrification inhibitor. For example, the volatility of N-Serve may lead to higher requirements in the field than in the laboratory where diffusion is restricted; thiourea would not be affected in this manner.

We have determined the effect of thiourea on nitrification by soil incubation experiments. Table I shows the rates of nitrate production from thiourea incubated alone with soil and from mixtures of thiourea with urea. These results show the same qualitative effects as McBeath's perfusion experiments, although the inhibition appears somewhat greater in the latter. Thus, at 21.5 mg of thio-

**Table I. Effect of Thiourea on Nitrification in Soil (mg/100 g of Soil)**

Urea N	Thio- urea	Thiourea N	$\text{NO}_3\text{N}$ produced at (weeks)		
			3	6	24
0	0	0	6.2	8.1	14.2
0	4.3	1.6	7.5	9.3	14.2
0	21.5	7.93	4.2	11.3	19.0
0	43	15.9	0.2	0.5	0.5
20	0	0	24.7	26.4	33.4
20	4.3	1.6	25.0	27.3	31.8
20	21.5	7.93	9.8	26.8	35.4
20	43	15.9	0.2	0.5	0.6

Hercules Incorporated, Research Center, Wilmington, Delaware 19899.

**Table II. Thiourea Solutions (Per Cent)**

Components	Thiourea (1)	With NH <sub>3</sub> (2)	With urea (3)	With NH <sub>3</sub> -urea (4)	With NH <sub>3</sub> -NH <sub>4</sub> NO <sub>3</sub> (5)	With NH <sub>3</sub> -NH <sub>4</sub> NO <sub>3</sub> -urea (6)
Thiourea	11.8	28	16	20.9	9.1	18
NH <sub>3</sub>		20		11.6	11.4	10
Urea			44	37.2		36
NH <sub>4</sub> NO <sub>3</sub>					50	10
H <sub>2</sub> O	88.2	52	40	30.2	29.5	26
Total N	4.4	26.9	26.5	34.7	30.2	35.2
Thiourea N	4.4	10.3	5.9	7.7	3.3	6.6
Salting out temp, °C	17-18	22	9	6-7	4-5	10-11

urea/100 g of soil, we found that the nitrate level reached that of the control within 6 weeks. In McBeath's experiments at 28 mg of thiourea/100 g of soil, more than 8 weeks was required. However, our 43 mg of thiourea showed almost complete inhibition of nitrate production, a point not yet reached at McBeath's 28-mg level. The perfusion technique has the effect of maintaining maximum efficiency of the thiourea, perhaps by keeping a larger portion of the soil in contact with the thiourea solution; at sufficiently high concentration the nitrification presumably would be completely inhibited, as in our incubation experiments.

**Thiourea Solutions.** Nitrification inhibitors are generally applied with solid fertilizers (Hauck and Koshino, 1971). However, the major portion of nitrogen fertilizers in this country is applied in the liquid form—anehydrous ammonia, aqua ammonia, and nitrogen solutions. Controlled release fertilizers or difficultly soluble nitrification inhibitors cannot be applied in the liquid form except as emulsions and suspensions which may involve handling and processing problems. Thus, direct application of a nitrification inhibitor in nitrogen solutions would be of interest.

Thiourea is soluble in water to the extent of about 12% at ordinary temperatures, giving a solution containing less than 5% nitrogen, compared with 82% for anhydrous ammonia and 25-35% for low-pressure nitrogen solutions. Thiourea is much more soluble in anhydrous ammonia; at 30°, 100 g of a saturated solution in anhydrous ammonia contains 64.9 g of thiourea; at -10°, the thiourea content is 50 g (Seidell, 1941). These solutions contain 53-60% of nitrogen of which 31-45% is thiourea nitrogen. Such solutions would have high vapor pressures of ammonia, and loss of ammonia could cause thiourea to crystallize, leading to plugging of lines and variations in concentration. Thiourea can also be dissolved in concentrated ammonium hydroxide to give a solution high in thiourea and acceptably high in nitrogen. For example, we dissolved 35 g of thiourea in 100 ml (90 g) of concentrated ammonium hydroxide. The solution contains 28% thiourea, 20% ammonia, and 52% water; it contains 26.9% nitrogen with 38.3% of the total as thiourea nitrogen. This aqueous solution thus has a proportion of thiourea nitrogen comparable to that in the anhydrous ammonia solutions, and it would be more convenient to use.

It is generally desirable to make nitrogen solutions as high in nitrogen content as possible, as tempered by need for the specific properties of individual nitrogen sources. The maximum nitrogen content is chiefly limited by the salting out temperature, the temperature at which solids separate from solution. This temperature should not be above temperatures ordinarily encountered, ca. 20° (68°F), and preferably should be 10° (50°F) or lower to avoid solids separating in tanks at night or in cool weather. With these requirements in mind and aiming at maximum thiourea concentration, we have prepared solutions from combinations of thiourea with ammonia, urea, and ammonium nitrate. Compositions and salting out temperatures of typical solutions are given in Table II. Both urea and

**Table III. Nitrification of Thiourea Solutions**

Solution	Thiourea N, mg/100 g of soil	% conversion of added N to nitrate at (weeks) <sup>a</sup>			
		3	6	9	15
2	7.9	-28.8	-7.3	59.5	70.0
3	4.5	9.3	82.0	94.5	94.0
4	4.6	0.05	74.5	92.7	93.4
5	3.2	20.4	65.0	90.1	89.6
6	3.9	46.7	85.8	100	100
Control	0	84.1	96	95	99
20 mg of N from (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>					

<sup>a</sup> Excludes nitrate from ammonium nitrate.

ammonium nitrate appear to exert a solubilizing effect on thiourea. The thiourea solutions with ammonia-urea-ammonium nitrate (solutions 3-6) range from 9 to 21% in thiourea and show reasonably low salting out temperatures. The thiourea-ammonia solution (solution 2) contains 28% thiourea but has a high salting out temperature (22°).

Production of nitrate from the thiourea solutions in Table II was determined by the incubation procedure used earlier to obtain results in Table I. Values are given in Table III.

In the combinations containing thiourea with ammonia and urea, alone or together (solutions 2-4, Tables II and III), a concentration of 4-8 mg of thiourea nitrogen per 100 g of soil required 3-6 weeks to produce nitrate at the same level as the soil control. This is in general agreement with the values indicated for solid thiourea in Table I. Solutions 5 and 6 contained nitrate originally; solution 5 showed greater inhibition than expected from its thiourea content, but solution 6 showed less inhibition than would be expected. All solutions showed significant inhibition after 3 weeks; after 6 weeks, only the thiourea-ammonia (solution 2) inhibition was pronounced, although the others were generally significantly lower than the ammonium sulfate control.

**Practical Considerations.** The experiments described indicate that thiourea will give rates of inhibition of practical interest at a concentration of 4-8 mg of thiourea nitrogen per 100 g of soil. To relate this quantity to pounds per acre, we can assume a weight of  $1 \times 10^6$  lb for the top 3 in. of an acre of soil (Buckman and Brady, 1960). Then the 4-8 mg/100 g of soil corresponds to 40-80 lb of thiourea nitrogen or 109-218 lb of thiourea per acre at the 3-in. depth. The assumption of the 3-in. layer is justified according to Smith (1966) who states: "Ammonia is attracted and held by the soil within the top 2 or 3 inches when applied in irrigation water." Perhaps the fertilizer-soil reaction zone is even narrower, which would make smaller amounts of thiourea effective. The use of thiourea at a 2% rate in fertilizer in Japan (Hauck and Koshino,

1971) suggests that thiourea may be effective in amounts smaller than indicated in the experiments cited. Fuller (1963) observed that thiourea at a rate of 50 lb of nitrogen per acre gave an increased barley yield over urea and other fertilizers. The considerations discussed are based on mixing thiourea with soil; broadcast application would approximate this, but placement or use in irrigation water would involve less soil per acre and would reduce the amount of thiourea required.

Hauck (1972) has pointed out that one of the requirements for a nitrification inhibitor is that it move with the fertilizer nitrogen in order to maintain an effective concentration throughout the soil-fertilizer reaction zone. The mobility of thiourea in soil has not been established, nor has the effect of the interaction of thiourea and ammonia on mobility been determined, but the polar nature and relatively low solubility of thiourea might be expected to cause it to remain close enough to adsorbed ammonia to preserve its effectiveness as a nitrification inhibitor.

In actual practice the utility of thiourea will depend on the amounts actually required under field conditions and on the cost of thiourea. At a large volume price of 10 cents per pound for thiourea, which appears possible, and at the lower levels indicated to be effective, the cost of using thiourea could approach costs for the more active nitrification inhibitors. The large amounts of thiourea required and its ease of application in solution would make it easy to avoid variations in rate, which would be an important advantage. Use with crops in the field is obviously required to complete the picture on thiourea as a practical nitrification inhibitor.

#### ACKNOWLEDGMENT

The authors are indebted to W. W. Haden and L. E. Anderson for the nitrification experiments.

#### LITERATURE CITED

- Buckman, H. O., Brady, N. C., "The Nature and Properties of Soils," Macmillan, New York, N. Y., 1960, p 53.  
 Clark, K. G., Yee, J. Y., Lundstrom, F. O., Lamont, T. G., *J. Ass. Offic. Agr. Chem.* 42, 596 (1959).  
 Fuller, W. H., *J. Agr. Food Chem.* 11, 188 (1963).  
 Fuller, W. H., Caster, A. B., McGeorge, W. T., *Ariz. Agr. Exp. Sta. Tech. Bull. No. 120*, 451 (1950).  
 Hauck, R. D., "Nitrification Inhibitors—Present Status and Future Use," Agronomy Abstracts, Annual Meeting of the American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Miami Beach, Fla., 1972, p 150.  
 Hauck, R. D., Koshino, M., in "Fertilizer Technology and Use," 2nd ed, Soil Science Society of America, Madison, Wis., 1971, pp 483-484.  
 Hays, J. T., Haden, W. W., Anderson, L. E., *J. Agr. Food Chem.* 13, 176 (1965).  
 McBeath, D. K., Ph.D. Thesis, Cornell University, Ithaca, N. Y., June, 1962 (reprint 62-5958, University Microfilms, Inc., Ann Arbor, Mich.).  
 Seidell, A., "Solubilities of Organic Compounds," D. Van Nostrand, New York, N. Y., 3rd ed, Vol. 2, 1941, p 43.  
 Smith, F. W., in "Agricultural Anhydrous Ammonia—Technology and Use," Agricultural Ammonia Institute, Memphis, Tenn., American Society of Agronomy, and Soil Science Society of America, Madison, Wis., 1966, p 116.

Received for review September 17, 1973. Accepted December 26, 1973. Presented at the Division of Fertilizer and Soil Chemistry, 166th National Meeting of the American Chemical Society, Chicago, Ill., Aug 1973. Hercules Research Center Contribution No. 1613.

## Biological Assessment of Available Iron in Food Products

Ezzat K. Amine<sup>1</sup> and D. M. Hegsted\*

A variety of fortified and unfortified food products were assayed for "available iron" using rat assays. It seems clear that the "curative assay," based upon the hemoglobin response of animals after prior iron depletion, is more satisfactory than the "prophylactic assay." Iron availability varied greatly and in some products fortified with ferric orthophosphate the added iron appeared to be essentially unavailable. In others, however,

ferric orthophosphate appeared to be a satisfactory form of iron. It seems apparent that the availability of iron in a food product may depend upon the form of iron added, the nature of the foodstuff fortified, and possibly the manufacturing process. Measurement of "available iron" by estimating the absorption of a small dose of <sup>59</sup>Fe added to the foodstuff did not appear to yield a satisfactory estimate of available iron.

The renewed interest in iron deficiency and the fortification of foods with iron has reemphasized the need for a method to determine the availability of iron in foods. The situation is complex since not only the kind of iron added to foods or the kind of iron in foods, but the nature of the food to which the iron is added and the foods consumed with the iron source influence the availability of the iron. Through the use of radioiron the differences in the availability of iron for human subjects in various sources have been clearly shown (Moore and Dubach, 1951; Hussain *et al.*, 1965; Layrisse *et al.*, 1969; Cook *et al.*, 1969). However, the difficulty of preparing labeled sources which duplicate commercial sources limits the utility of this ap-

proach as a routine method. The large intra- and inter-subject variability in iron absorption under apparently similar conditions also necessitates the use of relatively large groups of subjects if reasonably accurate estimates of the availability of iron in human subjects are to be obtained.

It is not clearly established how well the iron absorption in any animal species duplicates that in man, but many similarities are evident and it appears necessary to develop satisfactory animal assays for the routine testing of food products. Presumably, future comparative work will elucidate the relevance of such data to human nutrition.

The studies reported in this paper were done to further investigate the use and accuracy of the bioassay procedure previously reported (Amine *et al.*, 1972) for various iron preparations and fortified and unfortified food products. Comparative data were also obtained on the absorption of radioiron added to the diets containing these materials, *i.e.*, the extrinsic labeling of foods which has been pro-

Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts 02115.

<sup>1</sup>Present address: Department of Nutrition, High Institute of Public Health, Alexandria, Egypt.