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INHIBITION OF UREASE ACTIVITY IN SOILS

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Summary—Studies to evaluate more than 100 compounds as inhibitors of urease activity in soils showed that dihydric phenols and quinones were the most effective organic compounds tested and that silver and mercury salts were the most effective inorganic compounds. When the most potent inhibitors were compared using 50 μg of inhibitor per g of soil, their effectiveness decreased in the order: catechol > phenylmercuric acetate > hydroquinone > *p*-benzoquinone and 2,5-dichloro-*p*-benzoquinone > 2,6-dichloro-*p*-benzoquinone > silver sulfate > 1,2-naphthoquinone > phenol > mercuric chloride > sodium *p*-chloromercuribenzoate and 4-chlorophenol > 2,5-dimethyl-*p*-benzoquinone > N-ethylmaleimide > gold chloride > acetohydroxamic acid and copper sulfate. The inhibitory effects of most of these compounds on soil urease activity decreased markedly with time, but the effect of 2,5-dimethyl-*p*-benzoquinone increased, presumably because this compound is decomposed in soils to a relatively stable product more effective than the parent compound as a soil urease inhibitor. The findings concerning the rates of inactivation of urease inhibitors in soils indicate that, of the various compounds tested, 2,5-dimethyl-*p*-benzoquinone, 2,5-dichloro-*p*-benzoquinone, and 2,6-dichloro-*p*-benzoquinone are likely to prove the most effective for retardation of urea decomposition in soils and reduction of the problems caused by the normally rapid hydrolysis of fertilizer urea by soil urease. N-Serve and other compounds used to inhibit nitrification in soils have very little, if any, effect on soil urease activity.

INTRODUCTION

UREA is unique among commonly used synthetic nitrogen fertilizers in that its efficiency is greatly affected by a soil enzyme. In most soils, urea is rapidly hydrolyzed to ammonium carbonate through soil urease activity [$NH_2CONH_2 + 2H_2O \rightarrow (NH_4)_2CO_3$], and the resulting accumulation of ammonium and rise in pH can lead to several problems, including damage to germinating seedlings and young plants, nitrite toxicity, and gaseous loss of urea N as ammonia (see Gasser, 1964).

Cooke (1969) has drawn attention to the growing importance of urea as a fertilizer in world agriculture and to the need for research to overcome the problems encountered in use of this fertilizer. Attempts have been made to find compounds that will reduce these problems by effectively inhibiting urease activity in soils (Volk, 1961; Hyson, 1963; Moe, 1967; Tomlinson, 1967; Waid and Pugh, 1967; Anderson, 1969; Pugh and Waid, 1969a, 1969b; Sor, 1969), but they have been limited by the lack of a satisfactory method of evaluating such compounds. In most studies, evaluation has been performed by determining the effect of the test compound on ammonia evolution from soils treated with urea. Besides having defects pointed out by Pugh and Waid (1969b), this ammonia-volatilization test is too time-consuming for evaluation of a large number of compounds. This difficulty in research to find compounds having potential value as soil urease inhibitors has been overcome by the recent development of a rapid method of studying the abilities of different compounds to inhibit urease activity in soils (Douglas and Bremner, 1971). This method involves determination of the effect of the test compound on the amount of urea hydrolyzed by incubation of soils with urea (1000 ppm of urea N) and toluene at 37°C for 5 hr, and it is

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readily adapted for studies of the rates of inactivation of urease inhibitors in soils. The purpose of this paper is to report studies in which this method was used to evaluate more than 100 organic and inorganic compounds as inhibitors of urease activity in soils. The data reported permit direct comparison of the effectiveness of compounds thus far proposed as soil urease inhibitors, including compounds patented by Hyson (1963) and Anderson (1969).

MATERIALS AND METHODS

The soils used (Table 1) were surface (0- to 15-cm) samples used in previous work (Douglas and Bremner, 1971). Before use, they were air-dried and crushed to pass a 2-mm screen.

TABLE 1. ANALYSES OF SOILS

Soil No.	Type*	pH	Organic carbon %	Clay %	Sand %	CEC†	Urease activity‡
1	Fayette sicl	6·5	2·19	31	4	22·7	163
2	Nicollet l	7·0	2·27	20	44	19·2	248
3	Webster cl	7·3	3·03	31	28	33·0	283

* Sicl, silty clay loam; l, loam; cl, clay loam.
† Cation-exchange capacity (m-equiv./100 g of soil).
‡ μg of urea *N* hydrolyzed/g of soil/5 hr (Douglas and Bremner, 1971).

Acetohydroxamic acid was provided by Dr. W. N. Fishbein, 2-chloro-6-(trichloromethyl) pyridine (N-Serve) by the Dow Chemical Co., Midland, Michigan, 2-amino-4-chloro-6-methyl-pyrimidine (AM) by Toyo Koatsu Industries, Inc., Tokyo, and 2,4-diamino-6-trichloromethyl-*s*-triazine (CL 1580) by the American Cyanamid Co., Princeton, New Jersey. Urea phosphate was prepared as described by Gasser and Penny (1967). The other chemicals used were the highest-quality products available commercially.

The effects of different compounds on soil urease activity were studied by the 5-hr incubation test described by Douglas and Bremner (1971). This test involves determination of the effect of the test compound on the amount of urea hydrolyzed by incubation of 10-g samples of soils with 1 ml of toluene and 10 ml of urea solution containing 10 mg of urea N at 37°C for 5 hr, urea hydrolysis by soil urease being estimated by colorimetric determination of urea in the extracts obtained by treatment of the incubated soil samples with 2 M KCl containing a urease inhibitor (phenylmercuric acetate). Unless otherwise specified, the compounds evaluated as soil urease inhibitors were applied to soil; as aqueous solutions, and the rate of addition of each compound was equivalent to 50 ppm of soil.

All analyses reported represent the means of at least duplicate determinations.

RESULTS AND DISCUSSION

Evaluation of inorganic compounds

Studies of the effects of various metallic compounds on urease activity in Fayette and Webster soils showed that the inhibitory effects of 50 ppm (soil basis) of the metallic cations in these compounds decreased in the order: $Ag^+ > Hg^{2+} > Au^{3+} > Cu^{2+}, Cu^+ > Co^{2+}, Pb^{2+}, As^{3+}, Pb^+, Cr^{3+}, Ni^{2+}$ > others (Table 2). They also showed that, of the metallic salts tested, only those containing silver, mercury, gold, or copper had a substantial effect

on soil urease activity under the conditions of the 5-hr test. Comparison of the data for Cu^{2+}, Cu^{+}, Pb^{2+}, and Pb^{+} salts in Table 2 shows that the effects of these compounds on soil urease activity were not related to the valency state of their metal ions, and the results with Ag^{+}, Hg^{2+}, and Na^{+} chlorides and sulfates show that the anions in these compounds did not affect soil urease activity.

TABLE 2. EFFECTS OF METALLIC COMPOUNDS ON SOIL UREASE ACTIVITY

Compound	Metallic cation*	% Inhibition of urease activity	
		Soil 1	Soil 3
Silver nitrate	Ag^{+}	65	60
Silver sulfate	Ag^{+}	63	61
Cuprous chloride	Cu^{+}	16	14
Lead nitrate	Pb^{+}	3	2
Mercuric chloride	Hg^{2+}	42	38
Mercuric sulfate	Hg^{2+}	40	36
Cupric chloride	Cu^{2+}	16	13
Cupric sulfate	Cu^{2+}	14	15
Lead chloride	Pb^{2+}	4	4
Cobaltous chloride	Co^{2+}	4	6
Nickelous chloride	Ni^{2+}	1	2
Gold chloride ($HAuCl_4$)	Au^{3+}	18	20
Arsenic chloride	As^{3+}	4	2
Chromium chloride	Cr^{3+}	3	2
Others†	‡	0	0

* Rate of addition of each cation was equivalent to 50 ppm of soil.
† NaCl, Na_2SO_4, KCl, $CaCl_2$, $BaCl_2$, $ZnCl_2$, $MnCl_2$, $AlCl_3$, $FeCl_3$.
‡ Na^{+}, K^{+}, Ca^{2+}, Ba^{2+}, Zn^{2+}, Mn^{2+}, Al^{3+}, Fe^{3+}.

Volk (1961) and Pugh and Waid (1969a, 1969b) evaluated copper sulfate as a soil urease inhibitor by studying its effect on ammonia evolution from soils treated with urea. Volk's work showed that coating of urea fertilizer pellets with copper sulfate did not significantly decrease gaseous loss of urea N as ammonia from soils treated with these pellets and indicated that the copper sulfate treatment studied in his work had very little effect on soil urease activity. Pugh and Waid's results indicated that copper sulfate applied at the rate of 640 ppm of soil had a slight inhibitory effect on urease activity in several soils (see also Sor, 1969).

The following compounds had very little effect on soil urease activity when applied to soils 1 and 3 at the rate of 50 ppm of soil (the average percentage inhibition values obtained in the 5-hr test are given in parentheses): sodium azide (< 1 %), potassium dihydrogen phosphate (< 1 %), sodium sulfite (< 1 %), sodium bisulfite (4 %), arsenic trioxide (4 %), arsenic pentoxide (3 %), lead sulfide (3 %).

Studies in which H^{+} was added as H_3PO_4 and HNO_3 to soils 1–3 showed that the inhibitory effect of 50 ppm (soil basis) of H^{+} on soil urease activity was greater than that of 50 ppm of Cu^{2+} or Au^{3+}. The percentage inhibition values obtained in the 5-hr test were 27–32 % with phosphoric acid and 27–36 % with nitric acid.

The findings in these studies concerning the relative effects of different metal ions on soil

urease are similar to those in studies of the inhibitory effects of different metal ions on jackbean and soybean urease (see Shaw, 1954; Varner, 1960; Shaw and Ravel, 1961; Bahadur and Chandra, 1962; Toren and Burger, 1968). But our studies showed that the amounts of Ag^+, Hg^{2+}, and Cu^{2+} needed to effect substantial inhibition of soil urease are much greater than the amounts reported necessary for equivalent inhibition of jackbean or soybean urease (see Ambrose *et al.*, 1951; Shaw, 1954; Shaw and Ravel, 1961; Bahadur and Chandra, 1962; Toren and Burger, 1968). This is not surprising in view of the cation-exchange and metal-complexing properties of soils.

Evaluation of organic compounds

Table 3 shows results obtained in studies of the effects of sulphanilamide, dithiocarbamates, hydroxamates, and organic mercury compounds on soil urease activity.

TABLE 3. EFFECTS OF SULPHANILAMIDE, DITHIOCARBAMATES, HYDROXAMATES, AND ORGANIC MERCURY COMPOUNDS ON SOIL UREASE ACTIVITY

Compound*	% Inhibition of urease activity by compound		
	Soil 1	Soil 2	Soil 3
Sulphanilamide	<1	<1	<1
Sodium diethyldithiocarbamate	3	3	1
Dimethylammonium dimethyldithiocarbamate	7	7	3
Acetohydroxamic acid	16	14	13
Benzohydroxamic acid	12	10	8
Sodium *p*-chloromercuribenzoate	38	36	32
Phenylmercuric acetate	71	65	64

* Rate of addition of each compound was equivalent to 50 ppm of soil.

Pugh and Waid (1969a, 1969b) studied the effects of sulphanilamide, sodium diethyldithiocarbamate, acetohydroxamic acid, and benzohydroxamic acid on soil urease activity by the ammonia-volatilization technique and concluded that the effectiveness of these compounds as soil urease inhibitors decreased in the order: acetohydroxamic acid > benzohydroxamic acid > sodium diethyldithiocarbamate > sulphanilamide. The data in Table 3 confirm this conclusion.

Hyson (1963) patented metallic, ammonium, and dimethylammonium salts of alkyl and dialkyl dithiocarbamic acids as inhibitors of urease activity in soils (see also Tomlinson, 1967), but his patent did not report results obtained with these compounds or indicate how the dithiocarbamates patented were evaluated. The dithiocarbamates tested in our work (sodium diethyldithiocarbamate and dimethylammonium dimethyldithiocarbamate) were the only compounds patented by Hyson that could be obtained commercially. As Table 3 shows, they gave less than 8 per cent inhibition of soil urease activity under the conditions of the 5-hr test.

Several investigations (e.g. Kobashi *et al.*, 1962; Kornberg and Morris, 1962; Fishbein *et al.*, 1965; Pianotti *et al.*, 1966; Fishbein, 1967; Hase and Kobashi, 1967) have shown that acetohydroxamic acid and other hydroxamic acids are specific, noncompetitive inhibitors of bacterial and plant urease. Pugh and Waid (1969a, 1969b) evaluated aceto-, propiono-, capro-, caprylo-, benzo-, salicylo-, and phenylaceto-hydroxamic acid as soil urease inhibitors

by the ammonia–volatilization technique (see also Waid and Pugh, 1967). Their results indicated that only aceto- and benzo-hydroxamic acid are markedly effective as soil urease inhibitors and that, with most soils, acetohydroxamic acid is considerably more effective than benzo hydroxamic acid.

Table 3 shows that the two organic mercury compounds studied (phenylmercuric acetate and sodium *p*-chloromercuribenzoate) were considerably more effective than acetohydroxamic acid for inhibition of urease activity in soils. Phenylmercuric acetate was tested because previous work in our laboratory showed that this compound completely inhibited urea hydrolysis by soil urease during extraction of soils with 2 M KCl (Douglas and Bremner, 1970). The results with sodium *p*-chloromercuribenzoate confirm Moe's (1967) finding that this compound markedly inhibits soil urease activity. Moe (1967) found that *p*-chloromercuribenzoate applied at the rate of 115 ppm of soil inhibited nitrification as well as urease activity in two soils studied and did not decrease gaseous loss of urea N as ammonia following treatment of these soils with urea.

Studies with the following urea derivatives showed that none of them had a substantial effect on soil urease activity when applied to soils 1–3 at the rate of 50 ppm of soil (the average percentage inhibition values obtained in the 5-hr test are given in parentheses): urea phosphate (1%), urea nitrate (< 1%), urea oxalate (< 1%), biuret (2%), hydroxyurea (2%), phenylurea (4%), guanylurea sulfate (5%), thiourea (7%). The small inhibitory effects observed with urea phosphate, urea nitrate, and urea oxalate can be attributed to the acids in these compounds because tests previously discussed showed that acids depress urease activity in soils. Thiourea and hydroxyurea have been found to inhibit jackbean urease (Kistiakowsky and Shaw, 1953; Gale, 1965).

Studies with 17 compounds patented as inhibitors of nitrification in soils showed that, when applied to soils 1–3 at the rate of 50 ppm of soil, only one of these compounds (CL 1580) had a measurable effect on soil urease activity under the conditions of the 5-hr test (the percentage inhibition observed in this test was 1–2 per cent with CL 1580 and less than 1 per cent with the other compounds studied). The compounds tested were: 2-amino-4-chloro-6-methyl-pyrimidine (AM), sulfathiazole (ST), 2,4-diamino-6-trichloromethyl-*s*-triazine (CL 1580), 2-chloro-6-(trichloromethyl)-pyridine (N-Serve), *o*-nitroaniline, *m*-nitroaniline, *p*-nitroaniline, *o*-chloroaniline, *m*-chloroaniline, *p*-chloroaniline, 2-aminopyridine, 2-chloropyridine, 3-chloroacetanilide, *m*-nitroacetanilide, N-nitrosodimethylamine, N-methyl-N-nitrosoaniline, and 2-methyl-3-butyn-2-ol. AM and ST have been patented as nitrification inhibitors by Toyo Koatsu Industries, Inc., Tokyo, and CL 1580 has been patented by the American Cyanamid Co., Princeton, New Jersey. The other nitrification inhibitors tested have been patented by the Dow Chemical Co., Midland, Michigan.

Table 4 shows results obtained in studies of the effects of various phenolic compounds on soil urease activity.

The data for monohydric phenols in Table 4 show that, of the 13 compounds tested, only phenol and 4-chlorophenol gave marked inhibition of soil urease activity. Experiments with soils 1–3 showed that, when applied at the rate of 50 ppm of soil, 1-naphthol gave less than 2 per cent inhibition of soil urease activity under the conditions of the 5-hr test.

Anderson (1969) patented polyhydric phenols as inhibitors of urease activity in soils and reported tests in which catechol and hydroquinone were evaluated by studying their effects on ammonia volatilization from soils treated with urea. Table 4 shows that, of the 7 polyhydric phenols tested in our work, only catechol and hydroquinone had marked effects on soil urease activity.

Since the experiments reported in Table 4 showed that several phenolic compounds

TABLE 4. EFFECTS OF PHENOLIC COMPOUNDS ON SOIL UREASE ACTIVITY

Compound*	% Inhibition of urease activity by compound		
	Soil 1	Soil 2	Soil 3
Monohydric phenols:			
Phenol	43	41	41
4-Chlorophenol	37	37	30
Others (11)†	<1	<1	<1
Polyhydric phenols:			
Catechol	77	71	73
Hydroquinone	69	63	60
Pyrogallol	6	5	2
Others (4)‡	<4	<3	<2

* Rate of addition of each compound was equivalent to 50 ppm of soil.
† 2,5-dichlorophenol, 2-nitrophenol, 4-nitrophenol, 2,4-dinitrophenol, 2,4,6-trinitrophenol (picric acid), 4-chloro-2-nitrophenol, 4-hydroxybenzoic acid, 2-aminophenol, 4-aminophenol, 2-methoxyphenol (guaiacol), 4,6-dinitro-2-methoxyphenol.
‡ Resorcinol, protocatechuic acid, phloroglucinol, gallic acid.

have marked inhibitory effects on urease activity in soil, we studied the effects of lignins, tannins, and humic acids (polyphenolic substances) on soil urease activity. To evaluate these high-mol. wt. substances as soil urease inhibitors by the 5-hr test, it was necessary to apply them as solids because their low solubility in water did not allow application in aqueous solution at the rate of 50 ppm of soil. Tests were performed with soils 1 and 3, and the substances tested included 2 commercial lignin preparations (Indulin A and Meadol MRM), 5 commercial tannin preparations (quebracho, sumac, mimosa wattle, myrabalm, and chestnut), and 3 humic acid preparations (isolated from Iowa surface soils by the customary alkali and acid treatments). The results showed that none of the substances tested gave more than 4 per cent inhibition of soil urease activity under the conditions of the 5-hr test.

A study of the effects of various quinones and substituted quinones on urease activity in soils showed that all compounds tested except 2,5-dihydroxy-*p*-benzoquinone had a marked inhibitory effect (Table 5). Anderson (1969) patented quinones and substituted quinones as soil urease inhibitors and reported ammonia-volatilization tests indicating that 2,5-dimethyl-*p*-benzoquinone is more effective than catechol or hydroquinone and that synergism occurs when this substituted quinone is applied to urea-treated soils in conjunction with catechol or hydroquinone. The data in Tables 4 and 5 do not confirm Anderson's (1969) conclusion that 2,5-dimethyl-*p*-benzoquinone is more effective than catechol or hydroquinone as a soil urease inhibitor, but this divergence is explained by studies reported in Table 8 showing that the inhibitory effect of 2,5-dimethyl-*p*-benzoquinone on soil urease activity increases with time (Anderson's tests to evaluate 2,5-dimethyl-*p*-benzoquinone involved incubation for several days, whereas the incubation time in the tests reported in Tables 4 and 5 was only 5 hr).

Studies with 12 miscellaneous organic compounds (benzene, anisole, veratrol, benzoic acid, vanillic acid, N-ethylmaleimide, maleic acid, citraconic acid, phthalimide, iodoacetic acid, iodoacetamide, potassium ethylxanthate) showed that, when applied to soils 1–3 at the rate of 50 ppm of soil, only one of these compounds (N-ethylmaleimide) gave more than 4 per cent inhibition of soil urease activity under the conditions of the 5-hr test. The

TABLE 5. EFFECTS OF QUINONES AND SUBSTITUTED QUINONES ON SOIL UREASE ACTIVITY

Compound*	% Inhibition of urease activity by compound		
	Soil 1	Soil 2	Soil 3
p-Benzoquinone	68	61	58
2,5-Dichloro-*p*-benzoquinone	68	62	56
2,5-Dimethyl-*p*-benzoquinone	35	33	29
2,5-Dihydroxy-*p*-benzoquinone	7	6	6
2,6-Dichloro-*p*-benzoquinone	63	60	52
1,2-Naphthoquinone	48	42	42

* Rate of addition of each compound was equivalent to 50 ppm of soil.

inhibition values obtained with N-ethylmaleimide were 28% (soil 1), 24% (soil 2), and 23% (soil 3). Three of the compounds tested (N-ethylmaleimide, iodoacetic acid, and iodoacetamide) inhibit some enzymes containing sulfhydryl groups (Webb, 1966). Urease contains sulfhydryl groups and is inactivated by several reagents that react with such groups (see Varner, 1960).

Studies with most effective inhibitors

Table 6 shows the averages of the results obtained with soils 1–3 when the most effective of the various organic and inorganic inhibitors studied were compared using 50 μg of inhibitor per gram of soil. The data show that, when compared at the 50 ppm level, the effectiveness of these inhibitors decreased in the order: catechol > phenylmercuric acetate > hydroquinone > *p*-benzoquinone and 2,5-dichloro-*p*-benzoquinone > 2,6-dichloro-*p*-benzoquinone > silver sulfate > 1,2-naphthoquinone > phenol > mercuric chloride > sodium *p*-chloromercuribenzoate and 4-chlorophenol > 2,5-dimethyl-*p*-benzoquinone > N-ethylmaleimide > gold chloride > acetohydroxamic acid and copper sulfate.

The finding that the inhibitory effects of 50 ppm of silver sulfate, mercuric chloride, gold chloride, or copper sulfate on soil urease activity were less than those of 50 ppm of catechol, hydroquinone, or *p*-benzoquinone (Table 6) is noteworthy, because preliminary work to evaluate inorganic and organic compounds as urease inhibitors in the absence of soil showed that small amounts of Ag, Hg, Au, or Cu salts had a greater inhibitory effect on urea hydrolysis by jackbean urease than did the same amounts of catechol, hydroquinone, or *p*-benzoquinone. For example, we found that treatment of a urea solution with 10 ppm (solution basis) of silver sulfate or mercuric chloride gave almost complete (>99 per cent) inhibition of urea hydrolysis by jackbean urease, whereas 10 ppm of catechol, hydroquinone, or *p*-benzoquinone gave less than 70 per cent inhibition. The ability of soils to deactivate small amounts of urease inhibitors is evidently greater for inorganic inhibitors such as Ag and Hg salts than for organic inhibitors such as catechol, hydroquinone, and *p*-benzoquinone.

A study of the effects of different amounts of various inhibitors on the urease activity of Webster soil (no. 3) showed that none of the inhibitors studied gave complete inhibition of urease activity even when the rate of inhibitor addition was equivalent to 300 ppm of soil (Table 7). The percentage increase in inhibition effect when the level of inhibitor was increased from 50 to 300 ppm was greatest with the compound having the smallest effect at the 50 ppm level (acetohydroxamic acid).

TABLE 6. COMPARISON OF ORGANIC AND INORGANIC COMPOUNDS AS INHIBITORS OF SOIL UREASE ACTIVITY

Compound*	% Inhibition of urease activity Range	Average
Organic:		
Catechol	71–77	74
Phenylmercuric acetate	64–71	67
Hydroquinone	60–69	64
p-Benzoquinone	58–68	62
2,5-Dichloro-*p*-benzoquinone	56–68	62
2,6-Dichloro-*p*-benzoquinone	52–63	58
1,2-Naphthoquinone	42–48	44
Phenol	41–43	42
Sodium *p*-chloromercuribenzoate	32–38	35
4-Chlorophenol	30–37	35
2,5-Dimethyl-*p*-benzoquinone	29–35	32
N-Ethylmaleimide	23–28	25
Acetohydroxamic acid	13–16	14
Inorganic:		
Silver sulfate	45–52	48
Mercuric chloride	35–39	37
Gold chloride	17–19	18
Copper sulfate	13–14	14

* Rate of addition of each compound was equivalent to 50 ppm of soil.

TABLE 7. EFFECTS OF DIFFERENT AMOUNTS OF UREASE INHIBITORS ON SOIL UREASE ACTIVITY*

Inhibitor	Amount of inhibitor added (ppm of soil) 50	100	200	300
	% Inhibition of soil urease activity			
Catechol	73	81	89	94
Phenylmercuric acetate	64	71	77	81
Hydroquinone	60	75	86	93
p-Benzoquinone	58	74	86	93
2,5-Dichloro-*p*-benzoquinone	56	75	81	87
2,6-Dichloro-*p*-benzoquinone	52	66	81	87
Mercuric chloride	39	45	56	61
2,5-Dimethyl-*p*-benzoquinone	29	33	39	42
Acetohydroxamic acid	13	24	33	44

* Webster soil (no. 3).

For valid appraisal of the results obtained by the 5-hr incubation test used to evaluate different compounds as soil urease inhibitors, it is necessary to have information concerning the rates of inactivation of these compounds in soils. Table 8 show results obtained when the 5-hr incubation test was adapted to study the rates of inactivation of the compounds showing marked inhibitory effects in this test. The adaptation involved incubation of soil samples with these compounds at 30°C for various times before incubation with urea and toluene at 37°C as in the 5-hr incubation test. Control experiments showed that the urease

TABLE 8. EFFECT ON SOIL UREASE ACTIVITY OF INCUBATING SOILS WITH UREASE INHIBITORS AT 30°C FOR VARIOUS TIMES*

Inhibitor	Soil No.	Time of incubation of soil with inhibitor (days) 0	3	7	14
		% Inhibition of soil urease activity			
Catechol	1	77	—	46	—
	2	71	—	44	—
	3	73	54	39	30
Hydroquinone	1	69	—	31	—
	2	63	—	25	—
	3	60	42	14	12
p-Benzoquinone	1	68	—	30	—
	2	61	—	32	—
	3	58	38	23	18
Phenylmercuric acetate	1	71	—	49	—
	2	65	—	46	—
	3	64	51	39	9
1,2-Naphthoquinone	1	48	—	28	—
	2	42	—	26	—
	3	42	28	16	8
Mercuric chloride	1	39	—	38	—
	2	37	—	36	—
	3	35	34	33	32
4-Chlorophenol	3	30	21	10	3
Phenol	1	43	—	18	—
	2	41	—	16	—
	3	41	28	16	8
Sodium *p*-chloromercuribenzoate	1	38	—	23	—
	2	36	—	21	—
	3	32	27	14	6
2,5-Dimethyl-*p*-benzoquinone	1	35	—	73	76
	2	33	—	72	—
	3	29	66	71	74
2,5-Dichloro-*p*-benzoquinone	1	68	—	56	—
	2	62	—	53	—
	3	56	—	49	—
2,6-Dichloro-*p*-benzoquinone	1	63	—	56	—
	2	60	—	51	—
	3	52	—	47	—
N-Ethylmaleimide	1	28	—	23	—
	2	24	—	22	—
	3	23	21	19	8
Acetohydroxamic acid	1	16	—	0	—
	2	14	—	1	—
	3	13	5	0	0
Copper sulfate	1	14	—	12	—
	2	14	—	12	—
	3	13	—	10	—

* 10-g samples of soil treated with water or with water containing 500 μg of inhibitor were incubated at 30°C in stoppered 8-oz French square bottles for time specified (amount of water added was equivalent to 50% of water-holding capacity of sample). Samples were then treated with 1 ml of toluene, 5 ml of a solution containing 10 mg of N as urea, and enough water to bring total volume of water to 10 ml, and were incubated at 37°C for 5 hr and subsequently analyzed for urea.

activities of soils 1–3 increased slightly when these soils were incubated (no inhibitor or urea added) at 30°C as in the experiments reported in Table 8 (the average percentage increase in soil urease activity was 0·4 per cent after 3 days, 0·8 per cent after 7 days, and 1·8 per cent after 14 days).

The data in Table 8 show that the inhibitory effects of most of the compounds studied decreased markedly with increase in time of incubation of inhibitor-treated soil, but that the effect of 2,5-dimethyl-*p*-benzoquinone increased, presumably because this compound is decomposed in soils to a relatively stable product that is more effective than the parent compound as a soil urease inhibitor. The data for acetohydroxamic acid in Table 8 support Pugh and Waid's (1969a) conclusion that this compound is decomposed rapidly in soils and has no effect on urea hydrolysis by soil urease when it is added to soils several days before addition of urea (see also Waid and Pugh, 1967).

The findings concerning the rates of inactivation of urease inhibitors in soils indicate that, of the various compounds tested, 2,5-dimethyl-*p*-benzoquinone, 2,5-dichloro-*p*-benzoquinone, and 2,6-dichloro-*p*-benzoquinone are likely to prove the most effective for retardation of urea decomposition in soils and reduction of ammonia volatilization and other problems caused by the rapid hydrolysis of fertilizer urea by soil urease. Work in progress has confirmed that 2,5-dimethyl-*p*-benzoquinone greatly reduces gaseous loss of urea N as ammonia from soils treated with urea, but further studies with substituted quinones (including studies of their effects on plants and on nitrification in soils) are needed before any valid conclusions can be drawn about their potential practical value.

Besides being applied to soils as a nitrogen fertilizer, urea is added to many soils in the form of animal urine. Approximately 45 g of urea are voided daily by sheep and approximately 140 g are voided daily by mature cattle (Gasser, 1964). Where animals are confined to feedlots, the resulting heavy applications of urea as urine to small areas of soil lead to large accumulations of ammonium through urea hydrolysis by soil urease, and volatilization of this ammonium can cause an air-pollution problem. Moreover, studies by Hutchinson and Viets (1969) have indicated that ammonia evolved from cattle feedlots is absorbed by, and may promote eutrophication of, lakes and streams in the vicinity of these feedlots. The possibility that these problems can be reduced by application of urease inhibitors to feedlots clearly deserves attention.

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