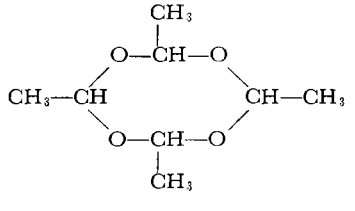
Colorimetric Determination of Metaldehyde Residues on Plants

PAUL A. GIANG and F. F. SMITH
Entomology Research Branch,
U. S. Department of Agriculture,
Beltsville, Md.

Metaldehyde powders and baits have attracted wide interest for the control of slugs and snails infesting vegetable and flower gardens. However, because metaldehyde is also toxic to warm-blooded animals, an analytical method is needed for its detection in plant materials. The method described involves extracting metaldehyde from the plant materials with chloroform, washing the extract first with cold 2% sodium bisulfite solution and then with ice water, converting the metaldehyde to acetaldehyde with steam distillation in the presence of trace of sulfuric acid, and estimating the acetaldehyde by Stotz's colorimetric procedure. With this method, sensitive to 0.5 γ of metaldehyde, the residues on Lima bean, geranium, and chrysanthemum plants were determined.

METALDEHYDE HAS ATTRACTED WIDE INTEREST for the control of slugs and snails infesting vegetable and flower gardens, since the report of its slug-killing properties by Hadden in 1936 (*4*). Both as a powder and as a bait metaldehyde can act on slugs either by contact or as a stomach poison (*2*, *3*, *8*). It has been suggested that this chemical causes desiccation of the slugs and poisoning of their nerve system (*3*, *5*). However, metaldehyde is also toxic to warm-blooded animals (*5*). In man it brings about fever, restlessness, and cramps, and in severe cases it will cause convulsive fits, stupor, and death [fatal oral dose, about 170 to 200 mg. per kg. (*9*)]. In view of this toxicity it was suggested that an analytical method be devised for the estimation of its residues on treated plants.

According to Pauling and Carpenter (*6*), metaldehyde is the tetramer of acetaldehyde containing an eight-membered ring:



As indicated in the formula, metaldehyde does not have a free aldehyde group in the molecule and, therefore, does not form a sodium bisulfite addition product or respond to any of the chemical tests for an aldehyde. It is practically insoluble in water, but is soluble in ethyl alcohol and in chloroform. Like the trimer, paraldehyde, metaldehyde can be converted easily into the monomolecular acetaldehyde by steam distillation in the presence of a small amount of sulfuric acid. There are many methods in the literature for the estimation of acetaldehyde. The method selected as most suitable and of sufficient sensitivity is that described by Barker and Summerson (*1*) for determination of lactic acid in biological material, and later adapted by Stotz (*7*) for estimation of acetaldehyde in blood. It is based upon the reaction of acetaldehyde in concentrated sulfuric acid solution with *p*-phenylphenol and a trace of cupric ions to form a brilliant violet color, which may be measured with a photometer.

The method described in this paper involves (1) chloroform extraction of the plant sample, (2) treatment of the chloroform solution with sodium bisulfite solution to remove any free acetaldehyde that may be present in the sample extract, (3) steam distillation of an aliquot of the chloroform solution in the presence of a small quantity of sulfuric acid to convert the metaldehyde into acetaldehyde, which in turn is absorbed in sodium bisulfite solution, (4) removal of the chloroform, and (5) development of the color by mixing an aliquot of the distillate with concentrated sulfuric acid which contains a trace amount of cupric sulfate and a solution of *p*-phenylphenol. The intensity of the color is measured in a photometer.

Method

Apparatus Photoelectric colorimeter, Klett-Summerson or equivalent. Nos. 56 and 54 green filters, spectral range 520 to 590 mμ.

Colorimetric tubes, matched.

Steam-distillation unit (all glass). A 100-ml. distillation flask with an adapter and a delivery tube attached to a 10-cm. Liebig sealed-type cold-water condenser by means of ball and socket joints. The delivery tube must be long enough to be dipped under the surface of 3 ml. of the 2% sodium bisulfite solution in a 150 × 15 mm. test tube used as the receiver. During the operation the receiver should be kept in an ice bath.

Reagents All reagents should be C.P. grade unless otherwise stated.

Chloroform, technical grade redistilled.

Sodium bisulfite ($Na_2S_2O_5$), 2% (freshly prepared).

Dilute sulfuric acid solution. Add 2 ml. of concentrated sulfuric acid to 500 ml. of distilled water.

n-Hexane, redistilled.

Sulfuric acid–cupric sulfate reagent. Mix 5 ml. of a 5% solution of cupric sulfate pentahydrate with 500 ml. of sulfuric acid. Store this solution in and dispense from an automatic buret equipped with a greaseless stopcock.

p-Phenylphenol reagent. Dissolve 1 gram of the crystalline material in 25 ml. of hot 2*N* sodium hydroxide and before cooling add 75 ml. of water. This reagent is stable for several months if stored in a brown bottle.

Metaldehyde, recrystallized from ether and ethyl alcohol.

Preparation of Standard Curves Weigh accurately 100 mg. of the recrystallized metaldehyde, transfer into a 200-ml. volumetric flask, and dilute to volume with chloroform. Dilute 20 ml. of this solution again to 200 ml. with chloroform. The second solution contains 50 γ of metaldehyde per milliliter of solution and is to be used as the working standard.

Remove free acetaldehyde as follows: Transfer the 200 ml. of the working standard solution into a 500-ml. separatory funnel, add 12 ml. (or 3 ml. for each 50 ml. of chloroform solution) of the freshly prepared, cold 2% sodium bisulfite solution, and shake the funnel for 1 minute. After separation of the layers,

transfer the chloroform layer into a second 500-ml. separatory funnel, and extract the chloroform solution four more times with 40-ml. portions of water (or 10 ml. for each 50 ml. of solution). Filter the chloroform solution through a thick plug of dry acetone-washed cotton into a glass-stoppered Erlenmeyer flask.

For the steam distillation pipet 3 ml. of the 2% sodium bisulfite solution into the test tube that is used as the receiver. Place the distillation flask in an ice bath, and introduce into the flask 10 ml. of the dilute sulfuric acid solution and the aliquot of metaldehyde standard solution to be tested. Add a clean quartz pebble. Dry the outside of the flask, and attach to the distillation unit. Distill slowly until about 3 to 4 ml. of the liquid remains in the distillation flask. Remove the flask, and rinse down the inside of the condenser with a little water. Then disconnect the delivery tube, and rinse the inside and outside with a little water.

Carefully decant the aqueous layer from the receiver into a 50-ml. volumetric flask. Wash the chloroform layer remaining in the receiver three times with 10 ml. of ice water each time by swirling. Make the solution and washings to the mark with water.

To remove the last traces of chloroform transfer the solution from the volumetric flask into a 125-ml. separatory funnel (without rinsing the flask), and add 5 ml. of *n*-hexane. Shake, and run the aqueous layer into a glass-stoppered Erlenmeyer flask. This is the solution to be used for the development of color.

To develop the color, place a 150 × 15 mm. borosilicate glass test tube containing 8 ml. of the sulfuric acid–cupric sulfate reagent in an ice bath. Pipet 1.0 ml. of the *n*-hexane–extracted solution into the test tube, and mix. Then add (with a medicine dropper) 4 drops of the *p*-phenylphenol reagent. Gently agitate the tube to disperse the *p*-phenylphenol suspension. Let the tube stand at 20° to 30° C. for 1 hour and then place it in a boiling-water bath for 90 seconds (*7*). Cool to room temperature. Measure the violet color in a photometer against a blank sample treated in the same manner. The color is stable for at least 2 hours at room temperature. Prepare the standard curves by plotting Klett-Summerson readings against micrograms of metaldehyde; these curves follow Beer's law, having slopes of 0.019 and 0.012 γ of metaldehyde per Klett-Summerson unit for filters 54 and 56, respectively.

Analysis of Plant Sample Extract a weighed amount of the chopped plant sample with a measured volume of the redistilled chloroform in a wide-mouthed glass jar on a tumbling machine for at least 1 hour. Use at least 200 ml. of solvent for each 100 grams of green plant material to ensure complete extraction. Filter the extract through a plug of dry acetone-washed cotton into a separatory funnel. Proceed with the analysis, starting with the treatment of the extract with 2% sodium bisulfite.

Tests Made with Method

The percentage recovery of metaldehyde added to young Lima bean plants was determined in the following manner: aliquots of a standard chloroform solution of metaldehyde were pipetted into a series of Mason jars containing a number of chopped and weighed (20 grams each) young plants. Each plant sample was extracted with 100 ml. of chloroform and analyzed by the procedure described. The results are shown in Table I.

Table I. Recovery of Metaldehyde from Young Lima Bean Plants

Added		Recovered		
γ	P.p.m.	γ	P.p.m.	%
252	12.6	238	11.9	94
		242	12.1	96
126	6.3	120	6.0	95
		120	6.0	95
63	3.2	60	3.0	95
		60	3.0	95
38	1.9	37	1.9	98
		36	1.8	95
			Average	95

A 15% metaldehyde dust was applied at the rate of 1 pound per 1000 square feet in a greenhouse in which the plants were growing in individual flower pots. The results obtained from the analyses of these plants are shown in Tables II and III. As shown in Table II, on the plants kept in the green house at hot summer temperatures, ranging from 37° to 44° C. on sunny days, the metaldehyde had almost completely disappeared in 24 to 48 hours, whereas on the plants kept in the mushroom house, at a temperature between 22° and 25° C., the metaldehyde was still detectable after 20 days. Table III gives the analytical results for metaldehyde on plants that had been treated in the same manner as those reported in Table II, but earlier in the year, when the temperature in the greenhouse was between 28° and 34° C., the metaldehyde lasted much longer on these plants. Plant materials that have been analyzed by the method include Lima bean plants and geranium and chrysanthemum leaves.

Discussion

When this colorimetric method was used for the determination of metaldehyde residues on plants, it was necessary prior to the steam distillation to treat the chloroform extract with 2% sodium bisulfite solution to remove any acetaldehyde and related materials. Elimination of this step invariably results in high readings as well as coloration of the reagent blank; moreover, green or light blue colors also developed from extracts of plants that were not treated with metaldehyde.

Although no interference has thus far been encountered from blank runs on plant material not treated with metaldehyde, it is always important to run a control analysis on a sample of the untreated material.

This method as described is extremely sensitive, the measurable range of the standard curves being only from 0.5 to 8.0 γ of metaldehyde. Because of this high sensitivity certain precautions must be taken in order to avoid any high color readings and appearance of color in the blank samples. Barker and Summerson (*7*) found that chromic acid (from the cleaning mixture), lubricating grease, and even perspiration from the hand caused erroneous results. The authors' experiences indicate that the breath used in blowing the reagent from a pipet into the test solution will give a green or blue color equivalent to as much as 1 γ of metaldehyde. On the other hand, chlorophyll and other colored substances extracted from plant materials as outlined in the procedure do not interfere with this method. Furthermore, incomplete removal of the chloroform from the aqueous solution inhibits the development of the color. Therefore, after steam distillation the chloroform distilled over must be removed by both washing with water and extracting with *n*-hexane.

Filters 54 and 56 were used in this

Table II. Metaldehyde Residues on Young Plants

(Treated on July 5, 1955. Parts per million)

Days after Treatment	Lima Bean Plants		Chrysanthemum Leaves	
	Greenhouse	Mushroom house	Greenhouse	Mushroom house
0	68.2	...	288.1	...
1	30.0	175.9	52.3	657.1
3	Trace	141.0	Trace	503.6
6	...	71.4	...	243.2
9	...	31.5	...	137.3
13	...	23.3	...	38.9
15	...	16.4	...	30.3
20	...	9.4	...	21.5

Table III. Metaldehyde Residues on Young Plants in Greenhouse

(Treated on May 31, 1955. Parts per million)

Days after Treatment	Lima Bean Plants	Geranium Leaves
0	292.5	378.2
1	232.2	279.2
3	201.3	208.3
8	17.8	5.3
10	0.3	2.0

method in measuring the color developed by the test solution. No. 56 is closer to the wave-length peak for the violet-blue color, but with the Klett-Summerson colorimeter it can measure only from 0.5 to 4.0 γ of metaldehyde. A standard curve based on the color readings with No. 54 filter was also constructed, as with this filter amounts of metaldehyde up to 8 γ may be measured.

Literature Cited

(1) Barker, S. B., Summerson, W. H., *J. Biol. Chem.* **138,** 535 (1941).
(2) Barnes, H. F., Weil, J. W., *Ann. Appl. Biol.* **29,** 56 (1942).
(3) Cragg, J. B., Vincent, M. H., *Ibid.*, **39,** 392 (1952).
(4) Hadden, N. G., *Garden Illus.* **58,** 255 (1936).
(5) Martin, Hubert, "Guide to Chemicals Used in Crop Protection," p. 159, Canada Dept. Agr., 1953.
(6) Pauling, Linus, Carpenter, D. C., *J. Am. Chem. Soc.* **58,** 1247 (1936).
(7) Stotz, Elmer, *J. Biol. Chem.* **148,** 585 (1943).
(8) Thomas, D. C., *Ann. Appl. Biol.* **35,** 207 (1948).
(9) Tilemans, E., Dormal, S., *Parasitica (Gembloux)* **8,** 64 (1952).

Received for review November 25, 1955. Accepted February 17, 1956.

RUMINANT NUTRITION

Review of Utilization of Nonprotein Nitrogen in the Ruminant

WILLIS D. GALLUP

Department of Agricultural Chemistry, Oklahoma Agricultural Experiment Station, Stillwater, Okla.

The utilization of nonprotein nitrogen, and the relative value of such nitrogen in meeting part of the protein requirement of ruminants, were successfully demonstrated in a series of nitrogen balance trials and practical feeding tests. Urea in amounts to supply up to 50% of the total supplemental nitrogen in fattening rations and 25% in wintering rations was efficiently utilized by beef cattle. Carbohydrate feeds were essential for efficient utilization, and methionine supplements brought further improvement. Chronic toxicity was not encountered. Acute toxicity was produced by drenching with solutions of urea and by fasting the animals before offering urea-containing feeds.

THE DOMESTIC RUMINANT has been described, with emphasis on rumen function, as a processor of plant material in the production of food for man—a processor that uses as raw material a high proportion of coarse, fibrous plants not suited to the diet of other animals and employs fermentation in breaking down the structural parts of plants to liberate and concentrate the more valuable nutrients contained therein, adding to these nutrients growth factors not originally present, and utilizing from the over-all processes appreciable amounts of the by-products as a source of energy. With respect to nitrogen metabolism, processing costs to the animal approach the equivalent of 3.5 grams of body protein per 100 grams of dry matter intake, the exact amount depending on the nature and composition of the intake; and, for maintenance, there is a further nitrogen cost related to body size. These costs are made apparent in the fecal and urinary nitrogen excretion of ruminants fed low-nitrogen rations.

The possibility that the nitrogen costs involved in digestion and body maintenance and even including production might be covered in part by supplying as a substitute for protein in the ration nonprotein nitrogen in the form of urea was put to critical test with sheep and dairy heifers about 15 years ago (*7, 8, 11*). The original concepts developed in the interpretation of the results of these tests have not changed. Together with other chemical and bacteriological studies of rumen processes, these tests demonstrated with reasonable certainty the formation of protein from nonprotein nitrogen through the intervention of microorganisms inhabiting the rumen. Practical application of the results to livestock production has encouraged further investigation of the nutritional requirements of rumen bacteria as related to those of the host. Nitrogen as a requirement, however, and the compounds of nitrogen that are most efficient in meeting this requirement, continue to occupy first place among the list of nutrients being studied by most investigators. No less than 15 reviews of the subject have appeared in the past decade.

Protein supplements play a double role in the nitrogen nutrition of the ruminant. First of all, and perhaps of greater importance, they supply in a natural form the nitrogen needed for growth and multiplication of those rumen organisms responsible for the fermentation and disintegration of the ration as a whole. This role of protein has been observed time and again, and it was effectively demonstrated a few years ago by Burroughs and his associates (*2*) in a series of studies dealing with the enhanced nutritive value of corn cob rations effected by protein and mineral supplementation. Much of the recent research on nonprotein nitrogen as a protein substitute has stemmed from, or been influenced by, observations made in those studies and later ones which employed the artificial rumen technique (*1*).

Further, protein supplements ultimately supply in the form of amino acids the nitrogen needs of the animal itself. Protein that is insoluble, is bound into the structure of plant cells by inert material, or has been so altered by physical and chemical means that it resists breakdown by bacterial enzymes of the rumen, is probably resistant to enzymatic breakdown in the gastrointestinal tract as well and therefore is of limited nutritive value (*12*). The low digestibility of heat-treated protein attests to this fact. On the other hand, easily soluble protein such as casein is extensively degraded in the rumen at so rapid a rate that ammonia and other simple compounds of nitrogen accumulate in amounts in excess of the immediate needs of the rumen organisms (*10*). Such excess of ammonia, although some of it is absorbed and returned to the digestive tract by way of the saliva, must be considered for the most part as wasted