Residue Determination of a Dioxane Herbicide in Soil and Soybeans by High-Pressure Liquid Chromatography

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A method is described for the determination of residues of FMC 25213 [*r*-2-ethyl-5-methyl-*c*-5-(2-methylbenzyloxy)-1,3-dioxane] in soil, soybeans, soybean forage, and soybean hay. Residues of FMC 25213 are first extracted from the sample with methanol–water and subsequently partitioned in methylene chloride. After being partitioned with sodium bisulfite, the methylene chloride extract is concentrated and added to a silica gel clean-up column. Acid hydrolysis of FMC 25213 releases propionaldehyde, which is distilled into a solution of 2,4-dinitrophenylhydrazine to form the corresponding hydrazone. Quantitative determination of the hydrazone is made by reverse-phase high-pressure liquid chromatography using a UV detector operating at 336 nm. The lower limits of sensitivity of this method are 0.025 ppm for soil, 0.05 ppm for soybeans and soybean forage, and 0.25 ppm for soybean hay. Recoveries of FMC 25213 averaged 85% for soil, 96% for soybeans, 93% for soybean hay, and 86% for soybean forage.

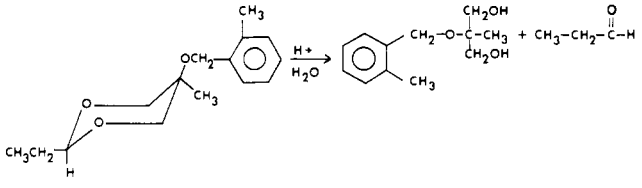
FMC 25213 [*r*-2-ethyl-5-methyl-*c*-5-(2-methylbenzyloxy)-1,3-dioxane] is a preemergence herbicide, effective in controlling annual grasses and certain broad-leaved weeds. This compound is being developed for the selective control of weeds in soybeans, peanuts, potatoes, tomatoes, and other dicotyledonous crops.

Although FMC 25213 can be detected by gas chromatography (GC) using nonspecific detectors, background interferences make this type of analysis at the residue level impractical. Trace amounts of FMC 25213 cannot be detected by specific GC detectors. Tagging the intact molecule to enhance detectability and selectivity is difficult, since there are no readily available active sites on the molecule. FMC 25213 does not absorb very strongly in the UV or visible regions. High-pressure liquid chromatography using a UV detector is thus not practical for detection of traces of FMC 25213. Greater sensitivity is needed to detect residue levels below 0.1 ppm.

Under acidic conditions, FMC 25213 breaks down to propionaldehyde and a diol as shown in Scheme I. Although a derivative of either the aldehyde or the diol can be prepared, a derivative of the aldehyde is more advantageous. The derivative of choice was the 2,4-dinitrophenylhydrazone (2,4-DNPH), since gas and liquid chromatographic conditions for the analysis of these derivatives of aldehydes and ketones have been investigated.

Soukup et al. (1964) and Fideli et al. (1965) separated a number of 2,4-DNPH of aldehydes and ketones by GC. The quantitative determination of formaldehyde in gross impure mixtures containing other carbonyl compounds was reported by Leonard and Kiefer (1966). Barrera et al. (1968) determined the carbonyl compounds in apples by making their, 2,4-DNPH derivatives and analyzing them by GC. A combination of thin-layer chromatography and gas chromatography was used by Shibasaki and Iwabuchi (1970) to determine the 2,4-DNPH derivatives of carbonyl compounds in miso aroma. Kallio et al. (1972) reported the GC separation of C_1–C_9 *n*-alkyl aldehydes as their 2,4-DNPH and suggested that an electron-capture detector was capable of detecting concentrations in the low picogram range. Papa and Turner (1972) improved the GC conditions to prevent decomposition of the derivatives in their chromatographic system and determined carbonyl compounds in auto exhaust as their 2,4-DNPH.

Scheme I



The major disadvantage in using GC for the analysis of the 2,4-DNPH of carbonyl compounds is the relatively low volatility of 2,4-dinitrophenylhydrazones of high molecular weight carbonyl compounds, necessitating operating the GC at high temperatures. This can cause some thermal decomposition of the derivatives. The 2,4-DNPH absorb in the UV and the visible regions and can be detected in low concentration by a spectrophotometric detector. Fitzpatrick et al. (1972) prepared 2,4-DNPH derivatives of 17 keto steroids. Henry et al. (1971) have detected as little as 10 ng of a 2,4-dinitrophenylhydrazone of keto steroid. Papa and Turner (1972) have detected carbonyl 2,4-DNPH down to 5 ng, and Carey and Persinger (1972) separated carbonyl 2,4-DNPH on HPLC. High-pressure liquid chromatography was chosen in preference to GC because HPLC was capable of separating the 2,4-DNPH of acetone and propionaldehyde.

EXPERIMENTAL SECTION

Reagents. Distilled in glass methanol, acetonitrile, and ethyl acetate (Burdick and Jackson Laboratories, Muskegan, Mich.), isooctane spectrograde and hydrochloric acid (Fisher, Pittsburgh, Pa.), anhydrous granular sodium sulfate (MCB, Norwood, Ohio), 2,4-dinitrophenylhydrazine (Analar, Carle Place, N.Y.), propionaldehyde (Eastman, Rochester, N.Y.), and sodium metabisulfite (Baker, Phillipsburg, N.J.) were used as received. Double distilled methylene chloride was also used. The silica gel, grade 923 (Grace, Baltimore, Md.), was adjusted to 5.5% moisture content before use. Analytical standards of FMC 25213 were supplied by the Agricultural Chemical Division, FMC Corporation, Middleport, N.Y.

Apparatus. The liquid chromatograph used was a Waters Associates Model ALC 202 equipped with a Valco Model CV-6-UH Pa-C-20 sample injection valve with a 50-μL loop; a 30 cm × 6 mm i.d. prepacked μ Bondapak

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C_{18} column (Waters Associates); and a Shoeffel SF 770 multiwavelength spectrophotometric detector operating at 336 nm. A Sorvall SS 3 automatic superspeed centrifuge, an Ohaus moisture balance, a twin shell blender, a grinding mill, a Hobart food chopper, a Millipore filter holder (47 mm, Pyrex Model XX 1004703), and Nucleopore filter pads (47 mm, 1 μm, GE) were also used.

Preparation of the 2,4-Dinitrophenylhydrazone of Propionaldehyde. The 2,4-dinitrophenylhydrazine reagent was prepared by dissolving 0.25 g of 2,4-dinitrophenylhydrazine in 100 mL of 6 N hydrochloric acid. The mixture was heated in a water bath, filtered if necessary, and stored in a brown bottle. The 2,4-DNPH of propionaldehyde was prepared by mixing equimolar amounts of propionaldehyde and the 2,4-dinitrophenylhydrazine solution at room temperature. The 2,4-DNPH of propionaldehyde precipitated immediately. The precipitate was filtered and recrystallized from methanol. The elemental composition (C, 45.38; H, 4.09; N, 23.79) agreed with the theoretical values (C, 45.38; H, 4.23; N, 23.52).

Sample Preparation. Soil samples were air-dried for 48 h and ground in a mill. Stones were removed by passing the sample through a no. 10 (2.0 mm) screen. The sieved samples were thoroughly homogenized in a twin shell blender. Samples of soybean forage and soybean hay were chopped in a Hobart food chopper, and soybeans were ground in the coffee mill attachment of the Hobart food chopper.

Extraction. (*a*) *Soil.* A soil sample (100 g dry weight) was placed in a blender jar with 150 mL of methanol and 75 mL of water. The sample was blended at high speed for 3 min and transferred to a 250-mL polypropylene centrifuge bottle. After centrifuging for 20 min at 6000 rpm, the water–methanol extract was decanted into a graduated cylinder and the volume was recorded. The water–methanol solution was filtered through a 1 μm Nucleopore filter using a Millipore filter holder connected to a 500-mL Erlenmeyer flask and a water aspirator.

(*b*) *Soybeans, Soybean Forage, and Soybean Hay.* FMC 25213 was extracted from soybeans and soybean forage by blending 50 g of sample with 200 mL of methanol and 100 mL of water at high speed for 3 min. The mixture was filtered through a Buchner funnel into a 500-mL Erlenmeyer flask and the volume recorded. Celite 545 was added to the filter paper to accelerate filtration. The volume of the filtrate was measured. In the case of soybean hay, a 10-g sample was blended with 80 mL of methanol and 40 mL of water using the conditions described above for soybean forage.

Cleanup. The methanol–water filtrate was transferred in a 1 L separatory funnel and extracted twice with 100 mL of methylene chloride. After being combined, the methylene chloride extact was extracted twice with 50 mL of a 2% solution of sodium bisulfite. The methylene chloride solution was then dried over anhydrous sodium sulfate, filtered, and concentrated to approximately 5 mL using a rotary evaporator. A clean-up column was prepared by placing a glass wool plug at the lower end of a 20 mm i.d. × 600 mm long glass tube and successively adding 3 g of sodium sulfate, 10 g of silica gel (5.5% moisture), and 3 g of sodium sulfate. The column was pre-wet with 25 mL of methylene chloride before the concentrated methylene chloride extract was added to the column. The methylene chloride was allowed to elute dropwise. When the methylene chloride reached the top of the sodium sulfate, 150 mL of a 1% solution of ethyl acetate in methylene chloride was added to the column. The next 50 mL of eluate was discarded, and the final 100 mL of eluate was collected in a 250-mL round-bottomed flask. The retained eluate was concentrated to approximately 5 mL using a rotary evaporator before being taken to dryness with a gentle stream of nitrogen.

Derivatization. Approximately 50 mL of 0.01 N hydrochloric acid was added to the round-bottom flask which was connected to a condenser through a U-shaped tube. A 250-mL Erlenmeyer flask fitted with a 24/40 ground glass joint served as the distillation receiver. Ten milliliters of isooctane and 0.1 mL of the 2,4-dinitrophenylhydrazine reagent were placed in the receiver. An adapter was used to connect the Erlenmeyer flask to the condenser. The delivery tube of the adapter was long enough to extend below the surface of the isooctane, providing a closed system. Distillation of the hydrochloric acid solution was started and continued until 25 mL of distillate had been collected. The receiver was removed, a magnetic stirring bar was placed in it, and the mixture was stirred at medium speed for 30 min. The mixture was then transferred to a separatory funnel and the isooctane phase collected in an Erlenmeyer flask. The aqueous phase was extracted with an additional 10 mL of isooctane. The isooctane extracts were combined and extracted twice with 20 mL of acetonitrile. The acetonitrile extract was concentrated to about 5 mL using a rotary evaporator and transferred to a graduated test tube. The acetonitrile was then concentrated to less than 2 mL using a gentle stream of nitrogen. The volume of the sample was adjusted to 2 mL and was then ready for HPLC analysis.

High-Pressure Liquid Chromatographic Analysis. Quantitative determination of FMC 25213 as the 2,4-DNPH of propionaldehyde was conducted using a high-pressure liquid chromatograph operating under the following conditions: column, μ Bondapak C_{18} (Waters Associates), 30 cm; column temperature, ambient; flow rate, 3 mL/min; detector, Shoeffel SF 770 spectrophotometer operating at 336 nm and 0.02 AUFS; pressure, about 1000 psi; chart speed, 0.2 in./min; mobile phase, acetonitrile–water (47:53). With the instrument equilibrated under these conditions, the retention time for the 2,4-DNPH of propionaldehyde was 9.0 min.

All calculations were based on a calibration factor obtained by dividing the amount of standard 2,4-dinitrophenylhydrazone of propionaldehyde injected (micrograms) by the peak height (millimeters) obtained. The calibration factor was obtained daily by chromatographing 0.1 and 0.2 μg of derivative. Since a plot of peak height vs. amounts of derivative injected is linear, a single calibration point in the form of a calibration factor is satisfactory for the calibration.

RESULTS AND DISCUSSION

Under acidic conditions, FMC 25213 hydrolyzes to propionaldehyde and a diol. Although it is possible to make a deivative of either fragment to enhance detectability by specific detectors, there are definite advantages to making a derivative of propionaldehyde.

The reaction of a hydrazine with an aldehyde to form the corresponding hydrazone is a more selective reaction than the standard reaction of an alcohol with an acid chloride to form an ester. Acid chlorides are not selective reagents and will react with many types of compounds besides alcohols, namely amines and phenols. The reaction between the hydrazine and the aldehyde offers the added advantage that it occurs at room temperature and in the presence of water. The reaction between the diol and the acid chloride is a more difficult reaction. Anhydrous conditions are required for esterification with an acid chloride, since water will compete for the acid chloride.

Table I. Conversion of Propionaldehyde to Its 2,4-Dinitrophenylhydrazone at Room Temperature in an Aqueous Reaction Medium

Time, h	μg added	μg found	% recov.
0.5	20	13.4	67
1	20	12.4	62
2	20	13.5	67.7
4	20	12.6	63.3
6	20	12.4	62.2
15	20	14.1	70.6

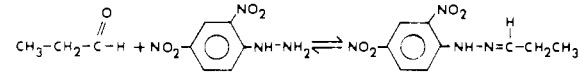
Heating is required for a quantitative reaction to occur between an acid chloride and an alcohol. The diol has two hydroxyl groups, both of which must be esterified to avoid formation of a mixture of derivatives, thus requiring prolonged heating. The formed diester, a bulky molecule, can be more difficult to gas chromatograph than the relatively simple hydrazone derivative.

Before hydrolyzing FMC 25213 to form propionaldehyde, naturally occurring aldehydes and methyl ketones, which could interfere with the analysis or give late eluting peaks can be selectively extracted with sodium bisulfite. No comparable selective removal of naturally occurring alcohols is available. After the hydrolysis of FMC 25213, propionaldehyde was separated from coextractants that remained by a simple distillation since it has a low boiling point of 40 °C. The diol has a high boiling point and has been found unstable after prolonged heating with acid. For these reasons, a derivative of propionaldehyde was chosen. The most commonly used hydrazone to determine traces of carbonyl compounds is the 2,4-dinitrophenylhydrazone, which can be detected by either GC or HPLC.

The 2,4-dinitrophenylhydrazone of propionaldehyde contains four nitrogens and can be analyzed by gas chromatography using either a nitrogen specific detector or an electron capture detector. A variety of gas chromatographic columns containing packings ranging in polarity from OV-1 to OV-25 was tried in efforts to separate the derivative of acetone from that of propionaldehyde, but without success. Since acetone is a very common contaminant of solvents, it could not be completely eliminated, even after elaborate precautions. Solvent checks, as well as soybean checks always gave an interferring peak varying between 0.02 to 0.05 ppm on the electron-capture and the Coulson conductivity detector. When HPLC was used, solent interference ranging between 0.02 to 0.05 ppm was present in the acetone 2,4-DNPH region, but no interference was observed in the propionaldehyde 2,4-DNPH region. Separation of acetone from propionaldehyde derivatives became a necessity to avoid interference from the 2,4-DNPH of acetone if a method sensitivity below 0.1 ppm was to be developed for routine analysis of FMC 25213.

A crop interference of 0.05 ppm was found in a few soybean samples. The silica gel column was introduced to remove this unidentified contaminant. After all contaminants, particularly carbonyl compounds, have been removed, FMC 25213 is hydrolyzed to propionaldehyde and a diol. The quantitative trapping of the propionaldehyde and its quantitative conversion to the derivative is critical for an accurate analysis of this volatile compound. Spiral condensers were found to be more efficient in condensing propionaldehyde than Liebig condensers.

The aqueous phase reaction between propionaldehyde and 2,4-dinitrophenylhydrazine was studied on a microscale at room temperature. Propionaldehyde was added to an excess of the 2,4-dinitrophenylhydrazine reagent. The solution was extracted at different time intervals and injected onto the HPLC. After 30 min, 67% of propionaldehyde was converted to the corresponding 2,4-dinitrophenylhydrazone. Allowing the reaction to proceed



for 15 h did not substantially improve the yield (Table I). These results indicate that an equilibrium was reached in the aqueous phase after 70.6% of the derivative was formed. When the same reaction is carried out with gram quantities, the 2,4-DNPH of propionaldehyde precipitates immediately because of its low solubility in the aqueous phase. The removal of the derivative from the aqueous phase by precipitation shifts the equilibrium toward the formation of more derivative and the reaction is almost quantitative. The water insolubility of the 2,4-DNPH of propionaldehyde indicated that the reaction could be quantitative if an aqueous–organic two-phase reaction was used. Propionaldehyde and the 2,4-dinitrophenylhydrazine reagent are water-soluble and react in the aqueous phase to form the derivative. The derivative is very organosoluble and will partition into the organic phase, shifting the equilibrium of the reaction toward the derivative and allowing the reaction to go to completion. Propionaldehyde was quantitatively converted to its 2,4-dinitrophenylhydrazone in 20 min (Table II).

Table II. Conversion of Propionaldehyde to Its 2,4-Dinitrophenylhydrazone at Room Temperature in a Two-Phase Reaction Medium

Time, min	μg added	μg found	% recov.
5	20	10.7	53.5
10	20	15.1	75.3
15	20	17.6	88.1
20	20	19.8	99
25	20	20	100.4
30	20	20	100.7

Table III. Recovery of FMC 25213 from Different Soils

Soil type	FMC 25213 added, ppm	Recov., %
Silt loam	0.025	82.8
Sandy loam	0.025	88.4
Loam	0.025	88.4
Loam	0.03	93.3
Sandy loam	0.035	80.0
Loamy sand	0.035	105.0
Clay	0.04	75.7
Sandy loam	0.05	74.4
Sandy loam	0.05	95.4
Silty clay	0.05	96.1
Loam	0.06	68.2
Clay	0.06	80.8
Loam	0.07	69.0
Clay	0.07	91.9
	Av	85

As shown in Tables III and IV, recoveries of FMC 25213 averaged 85% for soil, 94.1% for soybeans, 92.8% for soybean forage, and 85.8% for soybean hay. The method sensitivity for soil was 0.025 ppm, for soybean and soybean forage 0.05 ppm, and for soybean hay 0.25 ppm. The sensitivity of the method was limited by the sensitivity of the instrument and the practical size of the sample to be extracted.

Figure 1 shows a chromatogram of a standard of the 2,4-DNPH of propionaldehyde obtained by the procedure described. Representative chromatograms obtained from extracts of soil, soybeans, soybean forage, and soybean hay

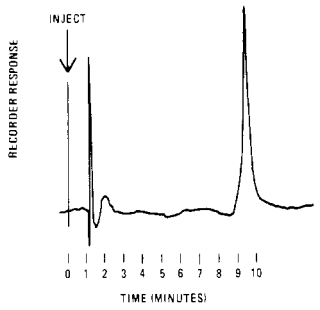


Figure 1. Liquid chromatograph of 100 ng of the 2,4-dinitrophenylhydrazone of propionaldehyde on 30 cm × 6 mm i.d. μ Bondapak C_{18}; mobile phase, 47% acetonitrile–water, flow rate, 3 mL/min; ambient column temperature; pressure about 1000 psi; Shoeffel UV Detector 336 nm; 0.02 absorbance full scale.

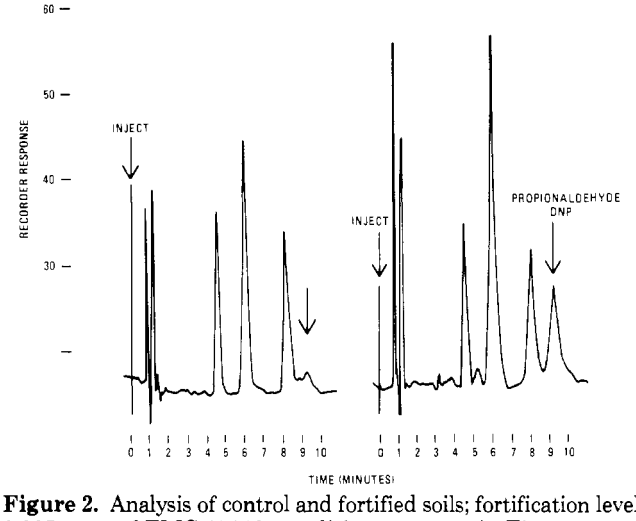


Figure 2. Analysis of control and fortified soils; fortification level 0.025 ppm of FMC 25213; conditions same as in Figure 1.

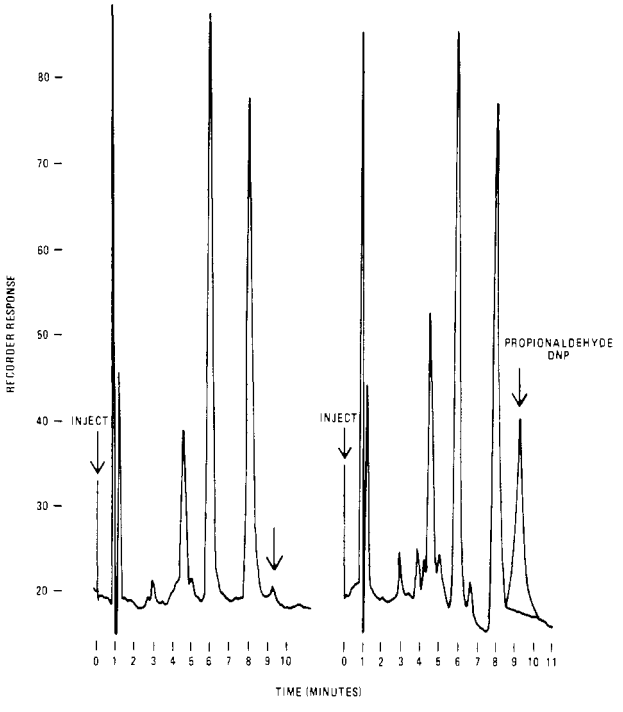


Figure 3. Analysis of control and fortified soybeans; fortification level of 0.1 ppm; conditions same as in Figure 1.

are shown in Figures 2 through 5. Three peaks eluting before the 2,4-DNPH of propionaldehyde are present in all these chromatograms. The peaks have a retention time corresponding to the 2,4-DNPH of formaldehyde, acetaldehyde, and acetone. None of the peaks interfered with

Table IV. Recovery of FMC 25213 from Soybeans, Soybean Hay, and Soybean Forage

Sample	FMC 25213 added, ppm	Recov., %
Soybeans	0.05	108.4
	0.05	102.4
	0.075	88.4
	0.075	89.3
	0.13	81.9
		Av 94.1
Soybean forage	0.05	99.2
	0.1	97.9
	0.1	81.2
		Av 92.8
Soybean hay	0.25	78.0
	0.25	91.2
	0.35	88.2
		Av 85.8

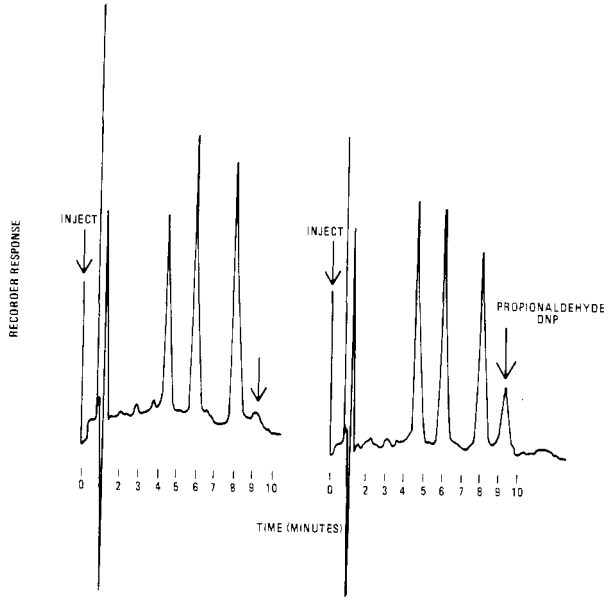


Figure 4. Analysis of control and fortified soybean forage; fortification level 0.1 ppm; conditions same as Figure 1.

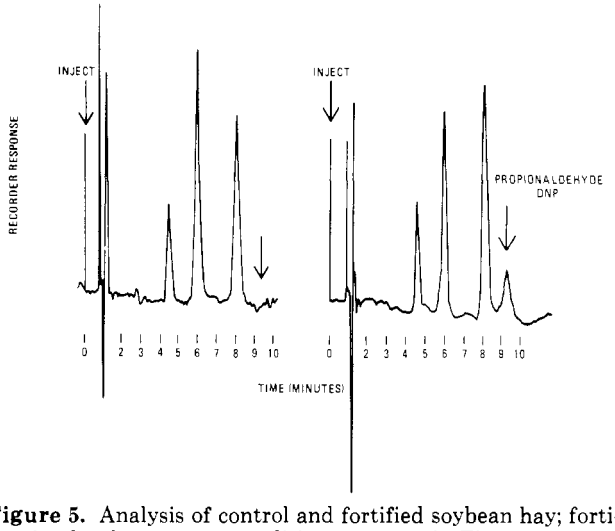


Figure 5. Analysis of control and fortified soybean hay; fortification level 0.25 ppm; conditions same as in Figure 1.

the 2,4-DNPH of propionaldehyde. No late eluting peaks were observed.

Gas chromatography can be used to analyze for FMC 25213 if a method sensitivity of 0.1 ppm is acceptable. Care must be taken to eliminate acetone from the solvents

used. This method is specific for the analysis of compounds that can be converted to a volatile aldehyde or ketone. FMC 25213 is an example of this type of compound since it hydrolyzes to propionaldehyde.

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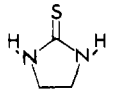
Oxidation of Ethylenebisdithiocarbamate Fungicides and Ethylenethiuram Monosulfide to Prevent Their Subsequent Decomposition to Ethylenethiourea

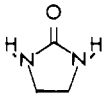
William D. Marshall

Oxidation of zineb in basic medium consumed approximately 16 equiv of hypochlorite and resulted in the production of 4 equiv of sulfate and 1 equiv of carbon dioxide but less than theoretical yield of ethyleneurea (EU), due in part to further oxidation of this product. Ethylenethiuram monosulfide (ETM) was oxidized by approximately 12 equiv of hypochlorite, which resulted in 3 equiv of sulfate and 0.45 equiv of EU. The degree of inactivation (to prevent subsequent decomposition to ethylenethiourea) of formulated zineb, maneb, mancozeb, and of ethylenethiuram monosulfide was found to be directly proportional to the quantity of oxidant added. The oxidative inactivation was equally effective for the three formulations and was complete in 5 min. No promotion of ETU formation was observed even when small quantities of oxidant were used. Hypochlorite oxidation in strong base is proposed as a technique for removal of residues of ethylenebisdithiocarbamates and ETM.

Ethylenebisdithiocarbamate (EBDC) fungicides (maneb, mancozeb, and zineb) provide broad spectrum control of fungal diseases and as such are widely used on a variety of crops. Concern for residues of these fungicides centers on the possibility that they may be converted to ethylenethiourea (ETU) (I), which is a potent tumorigen and teratogen to rats and mice (Graham et al., 1975; Graham, 1973; Graham and Hansen, 1972; Seiler, 1974; Khera, 1973; Ulland, 1972; Innis et al., 1969). Thus injestion of ETU over long periods of time may be harmful to human health.



ETU (I)



EU (II)

ETU formation from EBDCs is promoted by heat treatment (Marshall, 1977; Watts et al., 1974; Newsome and Laver, 1973) and has been demonstrated from EBDC field residues during normal food processing procedures (Baron, 1976). Thus a preprocessing technique whereby residues of these fungicides could be inactivated to avoid further decomposition to ETU would be of considerable utility.

The action of chloramine-T (p-$CH_3C_6H_4SO_2N$·NaCl) on hydrogen sulfide (Bendall et al., 1942; Murthy and Rao, 1952), on carbon disulfide (Rao and Murthy, 1960), and on zineb (Lakshminarayana, 1976) has been studied. The active agent of this oxidant in basic solution has been suggested to be hypochlorite (Afans'ev, 1948). The oxidation of aqueous sulfide solutions by hypochlorite has also been investigated (Choppin and Faulkenberry, 1937). These latter authors have demonstrated a complex reaction pathway in which the ratio of products formed (sulfate and elemental sulfur) was a function of the concentration of reactants, the temperture, and the pH of the reaction medium.

Recently it was reported that ETU is oxidized to ethyleneurea (II) and sulfate using hypochlorite, a potential preprocessing water wash additive (Marshall and Singh, 1977). This paper describes the action of hypochlorite on EBDC fungicides and on ethylenethiuram monosulfide (ETM) (III) and suggests optimal conditions under which this oxidation may be performed.

EXPERIMENTAL SECTION

Materials. Ethylenethiourea (ETU) (I) (2-imidazolidinethione) and ethyleneurea (II) (2-imidazolidinone) were purchased from Fisher Scientific Co. ETU was crystallized from methanol–water (1:1) containing 5% hexane, while EU was sublimed at 105 °C (10^{-3} mm) and recrystallized from methanol prior to use.

Nabam (disodium ethylenebisdithiocarbamate) was synthesized according to the method of Engst and Schnaak (1967), while zineb was obtained by adding nabam to aqueous zinc chloride (Ludwig et al., 1955) or by purifying

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