ERRATA NOTICE

Special Hazard Review with Control Recommendations
for
ETHYLENE THIOUREA
DHEW(NIOSH) Publication No. 79-109

Substitute the attached Appendix A for the Appendix A in the above mentioned book.

To be inserted in the above mentioned publication.

APPENDIX A

SAMPLING AND ANALYTICAL METHODS

There are a number of analytical techniques available in the literature which may be used to detect ETU in air, solutions, and solids. This multiplicity of methods has been largely due to the need of many investigators to detect minute amounts of ETU as contaminants or metabolic products of the ethylenebisdithiocarbamate fungicides.

The two main categories of detection have involved gas liquid chromatography (GLC), or wet chemistry (WC) methods, with GLC methods generally having greater sensitivity, specificity, and accuracy. However, most are best adapted for research rather than routine analysis.

The detectors utilized thus far in GLC have included electron capture (ECD), thermionic (TID), and flame photometric (FPD) detectors. While smaller sized samples may be used with the ECD, derivatization of the sample must usually be performed before detection. This step can be eliminated in the FPD method, but other FPD methods reported utilize derivatization which, of course, increases specificity. While selective GLC methods are more often used in research on ETU where confirmation of the identity of ETU is essential, it may also be used in routine analyses of air and solution samples as well. Solid samples nearly always require additional procedures.

In nearly all cases, the stepwise procedure for GLC analyses involves: (1) extraction, (2) cleanup, (3) derivatization, and (4) determination. Extraction is usually performed with methanol or ethanol but mixtures using acetone, chloroform, trichloroacetic acid and sodium ascorbate have also been used. Cleanup involves eliminating interfering substances. This is especially necessary in biological samples containing ETU. Cleanup is most efficiently done by separation of a derivative of ETU from an aqueous sample solution. Column chromatography or solvent partitioning may also be used (Onley and Yip, 1971).

In derivatization, the thio group is used for alkylation. This allows for the primary distinction among classes of chemicals similar to ETU. Various derivatives have been championed, each with various advantages. The final determination of the derivative is established by the partition coefficient along selected distinctive column packings.

Additional techniques for ETU analysis include: (1) thin layer chromatography (Vonk, 1970, Blazquez, 1973, Olney and Yip, 1971, and Engst, 1974)--this method generally has a lower detection limit of 20 ppb--; (2) pology raphy (Engst, 1974)--here the nitroso derivative is determined after alumina column cleanup--; (3) Radioactive derivatization (Nash, unpublished); (4) Spectographic (NIOSH standard method, Palassi, 1977). This simple, as yet unpublished spectophotometric method for the determination of ETU is especially suited to measurement of air samples. It has the combined advantages of direct reading, good sensitivity, and adequate specificity (all thio

groups). It is relatively fast and does not require expensive instrumentation. The NIOSH-verified method of analysis of ETU is presented below:

ETHYLENE THIOUREA

(2 Imidazoladinethione)

Measurements Support Branch

Analytical Method

Analyte:	Ethylene thiourea	Method No.:	P & CAM 281
Matrix:	Air	Range:	0.03-1.5 mg/m^3 for 100 l sample
Procedure:	Filter collection, extraction, complexation, spectrophotometry	Precision:	3% (Analytical)

1. Synopsis

Ethylene thiourea is collected from air on a PVC or cellulose ester membrane filter. The filter is extracted with distilled water. Pentacyanoammineferrate reagent is added to the extract to form a colored coordination

complex. The absorbance of the solution is measured spectrophotometrically at 590 nm, and the concentration of ethylene thiourea is determined from a calibration curve.

2. Working Range, Sensitivity, and Detection Limit

2.1 The working range for this method is 0.03-1.5 mg/m^3 in 100 l sample. The entire linear working range has not been determined.

2.2 The sensitivity is 0.006 absorbance units/μg, as determined from the slope of several calibration curves.

2.3 The detection limit is 0.75 μg/sample or 0.0075 mg/m^3 in 100 l sample for 0.01 absorbance, determined from the analysis of a 0.75 μg/sample, using 5 cm optical path length cells.

3. Interferences

3.1 Any compound which has an absorbance maximum in the region of the ethylene thiourea complex will interfere by causing a higher absorbance reading.

4. Precision and Accuracy

4.1 The analytical precision has been determined from 21 spiked PVC filters ranging from 15-150 μg/sample. The analytical relative standard deviation (RSD) is 3%.

4.2 The average percent recovery is 98.7% determined from 21 spiked PVC filters in the 15-150 μg/sample range.

5. Advantages and Disadvantages

5.1 The method is simple, relatively fast, has very good sensitivity and precision, and requires inexpensive instrumentation.

5.2 The pentacyanoammineferrate reagent will complex with compounds containing the thione (C=S) moiety; this method, therefore, will be applicable to those compounds containing this moiety.

5.3 The complexing reagent is stable for at least two weeks when refrigerated.

5.4 Stability studies of the ethylene thiourea complex revealed a 1.5% degradation of the color in 3 hours.

6. Apparatus

6.1 Air Sampling Equipment

6.1.1 Vinyl Metricel (VM-1) filters, 5 μm in pore size, 37 mm in diameter (Gelman Corp) or any equivalent filter.

6.1.2 Plastic three-piece 37 mm filter holders (cassettes) (Millipore Corp.) or eqivalent.

6.1.3 A personal air sampling pump capable of operating for 6 hours at 2.0 liters/min.

6.2 Visible Spectrophotometer.

6.3 Matched glass cells, 5 cm optical path length.

6.4 Analytical balance, capable of weighing to the nearest 0.1 mg.

6.5 Mortar and pestle.

6.6 Water bath, thermostatically controlled to ± 1 C.

6.7 Glass vials, 25 ml capacity, with teflon-coated screw caps.

6.8 Beakers, assortment of 50-250 ml.

6.9 Adjustable pipettes (5-50 µl, 50-250 µl, 0.5-5 ml) with disposable tips.

6.10 Volumetric pipettes, assortment of 1-25 ml capacity.

6.11 Volumetric flasks, 25, 100, 200, and 250 ml capacity.

6.12 Miscellaneous: tweezers, microspatula, rubber suction bulb, wood applicator stick, weighing paper, filter paper, and filter funnel with stand.

7. Reagents

All reagents used should be ACS reagent grade or better.

7.1 Bromine liquid, Br2. (Caution: corrosive liquid, causes severe burns, vapors extremely irritating and toxic. Wear gloves and handle only in a hood).

7.2 Sodium nitroferricyanide (sodium nitroprusside), $Na_2Fe(CN)_5NO \cdot 2H_2O$.

7.3 Hydroxylamine hydrochloride, $NH_2OH \cdot HCl$

7.4 Sodium bicarbonate, $NaHCO_3$

7.5 Hexane

7.6 Methanol

7.7 Distilled or deionized water

7.8 Complexing Reagent

7.8.1 Weigh 0.500 g sodium nitroferricyanide in a 50 ml beaker. Add 10 ml distilled water to the beaker and swirl to dissolve.

7.8.2 Weigh 0.500 g hydroxylamine hydrochloride and 1.000 g sodium bicarbonate. Transfer both materials to a mortar and carefully grind them together.

7.8.3 The next two steps must be performed in a hood. Add the ground mixture to the sodium nitroferricyanide solution. A bubbling reaction occurs for a few minutes. When the reaction is complete, add 0.10 ml bromine (see "caution" on Section 7.1). When effervescence stops, add approximately 10 ml distilled water and filter the solution. Rinse the beaker with 4 ml distilled water and filter. Transfer filtrate to a 25 ml volumetric flask and dilute with distilled water to the mark. This reagent should be kept refrigerated.

7.8.4 Dilute complexing reagent. Mix one part of complexing reagent with two parts water. Prepare this solution daily when needed.

7.9 Ethylene thiourea (2-imidazolidinethione). Usually this material is less than 99% pure and needs to be purified by recrystallization.

7.9.1 Recrystallization. In a 250 ml Erlenmeyer flask, weigh 3-5 g ethylene thiourea. Add 100 ml 1:1 methanol-water to dissolve the material. In a hood, heat the mixture to

boiling. Cool the flask at room temperature for five minutes. Add 5 ml hexane and shake the flask for 30 seconds. Cover the flask with a watch glass and leave undisturbed at room temperature for one hour. Filter the purified crystals, washing with 100 ml methanol-water mixture. Let the crystals air dry in the hood.

7.9.2 Caution: Ethylene thiourea has been found to be a teratogen and carcinogen in experimental animals. Extra care must be taken to avoid inhalation or skin contact with this material. Keep all material in a labeled bottle clearly identified as "potential human carcinogen," and place in a resealable thick-walled plastic bag, in locked storage.

7.10 Ethylene thiourea 1000 µg/ml stock solution.

7.10.1 Weigh 0.250 g of recrystallized ethylene thiourea in a beaker. Add distilled water to dissolve the material. Transfer the solution into a 250 ml volumetric flask and dilute with distilled water to the mark.

8. Procedure

8.1 Cleaning of Equipment

8.1.1 Wash all glassware with detergent solution, rinse with tap water, distilled water, and dry in an oven.

8.2 Calibration of sampling pump. The personal sampling pump should be calibrated with a representative filter assembly in the line. A wet or dry test meter or a glass rotameter capable of measuring the flow rate to within $\pm$5% may be used for the calibration.

8.3 Collection and Shipping of Samples

8.3.1 Assemble the filters in the filter holder so that the air being sampled passes first through the PVC filter and then through the filter support. Remove the small plugs from the filter holder and connect the filter holder to the sampling pump by means of an adapter and a length of tubing.

8.3.2 Sample at least 100 liters of air. The optimum sample volume will depend on the type of workroom environment being sampled. Since high concentrations of particulate material may plug the filter, the flow rate should be checked at least once every hour. On completion of sampling, reinsert the small plugs into the inlet and outlet of the filter holder. Record the temperature and pressure of the air being sampled.

8.3.3 With each group of samples prepare two blanks consisting of a filter holder with a representative filter that has been handled in the same manner as the sample filters, except that no air is drawn through them. The samples and the blank should be shipped promptly in a damage-proof container that allows no filter holder movement. Samples should be refrigerated as soon as possible.

8.4 Analysis of Samples

8.4.1 Extraction

1. Remove the top portion of the filter holder. Hold the bottom portion containing the filter and filter support over a piece of weighing paper to catch any particulate material that may fall out. Remove the small plug from the bottom portion of the filter holder and insert the applicator stick through the hole. Gently raise the filter support and the filter and grasp the unexposed edge with tweezers. Very carefully pick up the filter, insert it in a glass vial, and push it gently to the bottom with the applicator stick. Add to the vial any particulate material remaining in the filter holder or collected on the weighing paper.

2. Pipet 7.0 ml of distilled water into the vial. This amount should completely cover the filter. Screw the cap on the vial.

3. Place vials in a 60 C water bath (thermostatically controlled) for 45 minutes. The water bath level must be above the water level of the vial. Shake each vial every 5 minutes. The use of ultrasonic bath is not recommended because it breaks up the PVC filter.

4. Lift the filter with tweezers so it is above the water level in the vial and wash the filter eight times with 1 ml aliquots of water using a 1 ml pipette. Position the filter over the center of the vial so the rinsings can be collected in the vial, then discard the filter.

8.4.2 Complexation

This step should be performed at the same time for both standard and field samples so that the color formation will start at the same time. This reduces color degradation discrepancies.

1. Pipet a 1.5 ml aliquot of the <u>dilute</u> complexing reagent into each vial.

2. Allow the vials to stand for at least 30 minutes before analysis to insure full color development. Shake the vials every 10 minutes.

8.4.3 Analysis

1. Transfer the solution to a clean 5-cm optical path length glass cell. Wipe off with a lens paper any droplets left on the cell windows.

2. Place the cell in the sample compartment and measure the absorbance at 590 nm. The reference sample contains 15 ml distilled water and 1.5 ml dilute complexing reagent in a 5-cm glass cell. Record the absorbance for each sample.

8.5 Determination of Extraction Efficiency

8.5.1 The extraction efficiency of ethylene thiourea may vary from laboratory to laboratory. The average percent recovery determined from 21 spiked filter samples was 98.7% with a 3% relative standard deviation in the 15-150 µg/sample range.

8.5.2 Procedure

1. On a plastic test tube rack place eight PVC filters. Using an adjustable pipette, add to the center of each filter 0, 15, 30, 45, 60, 90, 120, and 150 microliters of the 1000 µg/ml ethylene thiourea stock solution. This corresponds to concentrations of 0, 15, 30, 45, 60, 90, 120, and 150 micrograms per filter. Let filters air-dry overnight at room temperature. Place each spiked filter in a vial, and mark its concentration. Follow sections 8.4.1 parts 2 through 8.4.3 for preparation and analyses of the spiked samples.

2. The absorbance of each sample is converted to micrograms from the calibration curve (Section 10.1). Percent recovery is determined as follows:

$$\text{Percent recovery} = \frac{\text{micrograms recovered}}{\text{micrograms added}} \times 100$$

9. Calibration and Standardization

9.1 Ethylene thiourea 15 µg/ml standard solution.

9.1.1 Pipet 3 ml aliquot of the 1000 µg/ml stock solution into a 200 ml volumetric flask. Dilute with distilled water to the mark.

9.2 Preparation of Standard Samples

9.2.1 Prepare the standards by following Table I. Pipet aliquots of the 15 μg/ml standard solution and distilled water in a marked vial.

TABLE I

Volume 15 µg/ml std	Volume distilled water	Concentration
ml	ml	µg/16.5 ml sample solution
0.00	15.00	0
1.00	14.00	15
2.00	13.00	30
3.00	12.00	45
4.00	11.00	60
6.00	9.00	90
8.00	7.00	120
10.00	5.00	150

Standards contain 1.5 ml of dilute complexing reagent. Total volume is 16.5 ml/vial. Follow section 8.4.2 and 8.4.3 to complex and analyze the standards.

9.2.2 Standards must be prepared and analyzed in conjunction with field samples. Analyses of standards should be performed before and after field samples are analyzed.

9.2.3 Prepare a calibration curve by plotting absorbance vs concentration of standards in µg/16.5 ml sample.

10. Calculations

10.1 The concentration of ethylene thiourea in field samples can be determined graphically from the plot of absorbance vs concentration.

The concentration can also be determined using the following expression obtained from least squares analysis.

$$\text{Concentration in } \mu g/\text{sample} = \frac{\text{Absorbance - y intercept}}{\text{slope}}$$

10.2 The concentration of ethylene thiourea in air may be expressed in mg/m^3.

$$mg/m^3 = \frac{\text{weight of field sample (in } \mu g)}{\text{volume of air sampled (in l)}}$$

10.3 For personal pumps with rotameters only, the following correction for air volumes sampled should be made:

$$\text{Corrected Volume} = f \times t \sqrt{\frac{P_1}{P_2} \times \frac{T_2}{T_1}}$$

where:

f = sample flow rate

t = sampling time

P_1= pressure during calibration of sampling pump (mm Hg)

P_2= pressure of air sampled (mm Hg)

T_1= temperature during calibration of sampling pump (K)

T_2= temperature of air sampled (K)

11. References

11.1 Grote WI: A New Color Reaction for Soluble Organic Sulfur Compounds. J Biol Chem 93:25, 1931

11.2 Danowski TS: Measurement of Thiourea in Ultrafiltrate of Serum. J Biol Chem 152:201, 1944

11.3 Association of Official Agricultural Chemists, 31, 100, 1948

APPENDIX A

SAMPLING AND ANALYTICAL METHODS

There are a number of analytical techniques available in the literature which may be used to detect ETU in air, solutions, and solids. This multiplicity of methods has been largely due to the need of many investigators to detect minute amounts of ETU as contaminants or metabolic products of the ethylenebisdithiocarbamate fungicides.

The two main categories of detection have involved gas liquid chromatography (GLC), or wet chemistry (WC) methods, with GLC methods generally having greater sensitivity, specificity, and accuracy. However, most are best adapted for research rather than routine analysis.

The detectors utilized thus far in GLC have included electron capture (ECD), thermionic (TID), and flame photometric (FPD) detectors. While smaller sized samples may be used with the ECD, derivatization of the sample must usually be performed before detection. This step can be eliminated in the FPD method, but other FPD methods reported utilize derivatization which, of course, increases specificity. While selective GLC methods are more often used in research on ETU where confirmation of the identity of ETU is essential, it may also be used in routine analyses of air and solution samples as well. Solid samples nearly always require additional procedures.

In nearly all cases, the stepwise procedure for GLC analyses involves: (1) extraction, (2) cleanup, (3) derivatization, and (4) determination. Extraction is usually performed with methanol or ethanol but mixtures using acetone, chloroform, trichloroacetic acid and sodium ascorbate have also been used. Cleanup involves eliminating interfering substances. This is especially necessary in biological samples containing ETU. Cleanup is most efficiently done by separation of a derivative of ETU from an aqueous sample solution. Column chromatography or solvent partitioning may also be used (Onley and Yip, 1971).

In derivatization, the thio group is used for alkylation. This allows for the primary distinction among classes of chemicals similar to ETU. Various derivatives have been championed, each with various advantages. The final determination of the derivative is established by the partition coefficient along selected distinctive column packings.

Additional techniques for ETU analysis include: (1) thin layer chromatography (Vonk, 1970, Blazquez, 1973, Olney and Yip, 1971, and Engst, 1974) --this method generally has a lower detection limit of 20 ppb--;(2) pology raphy (Engst, 1974) --here the nitroso derivative is determined after alumina column cleanup--; (3) Radioactive derivatization (Nash, unpublished); (4) Spectographic (NIOSH standard method, Palassi, 1977). This simple, as yet unpublished spectophotometric method for the determination of ETU is especially suited to measurement of air samples. It has the combined advantages of direct reading, good sensitivity, and adequate specificity (all thio

groups). It is relatively fast and does not require expensive instrumentation. The NIOSH-verified method of analysis of ETU is presented below:

ETHYLENE THIOUREA

(2 Imidazolinethione)

Measurements Support Branch

Analytical Method

Analyte:	Ethylene thiourea		
Matrix:	Air	Range:	0.03-1.5 mg/m3 for 100 l sample
Procedure:	Filter collection, extraction complexation, spectrophotometry	Precision:	3% (Analytical)

1. Synopsis

Ethylene thiourea is collected from air on a PVC membrane filter. The filter is extracted with distilled water. Pentacyanoamine-ferrate reagent is added to the extract to form a colored coordination

complex. The absorbance of the solution is measured spectrophotometrically at 590 nm, and the concentration of ethylene thiourea is determined from a calibration curve.

2. Working Range, Sensitivity, and Detection Limit

2.1 The working range for this method is 0.03-1.5 mg/m3 in 100 l sample. The entire linear working range has not been determined.

2.2 The sensitivity is 0.006 absorbance units/g, as determined from the slope of several calibration curves.

2.3 The detection limit is 0.75 g/sample or 0.0075 mg/m3 in 100 l sample for 0.01 absorbance, determined from the analysis of a 0.75 g/sample, using 5 cm optical path length cells.

3. Interferences

3.1 Any compound which has an absorbance maximum in the region of the ethylene thiourea complex will interfere by causing a higher absorbance reading.

4. Precision and Accuracy

4.1 The analytical precision has been determined from 21 spiked PVC filters ranging from 15-150 g/sample. The analytical relative standard deviation (RSD) is 3%.

4.2 The average percent recovery is 98.7% determined from 21 spiked PVC filters in the 15-150 g/sample range.

5. Advantages and Disadvantages

5.1 The method is simple, relatively fast, has very good sensitivity and precision, and requires inexpensive instrumentation.

5.2 The pentacyanoammineferrate reagent will complex with compounds containing the thione (C=S) moiety; this method, therefore, will be applicable to those compounds containing this moiety.

5.3 The complexing reagent is stable for at least two weeks when refrigerated.

5.4 Stability studies of the ethylene thiourea complex revealed a 1.5% degradation of the color in 3 hours.

6. Apparatus

6.1 Air Sampling Equipment

6.1.1 Vinyl Metricel (VM-1) filters, 5 um in pore size, 37 mm in diameter (Gelman Corp) or any equivalent filter.

6.1.2 Plastic three-piece 37 mm filter holders (cassettes) (Millipore Corp.) or equivalent.

6.1.3 A personal air sampling pump capable of operating for 6 hours at 2.0 liters/min.

6.2 Visible Spectrophotometer.

6.3 Matched glass cells, 5 cm optical path length.

6.4 - (deleted)

6.5 Mortar and pestle.

6.6 Water bath, thermostatically controlled to $\pm$1OC.

6.7 Glass vials, 25 ml capacity, with teflon-coated screw caps.

6.8 Beakers, assortment of 50-250 ml.

6.9 Adjustable pipettes (5-50 ml, 50-250 ml, 0.5-5 ml) with disposable tips.

6.10 Volumetric pipettes, assortment of 1-25 ml capacity.

6.11 Volumetric flasks, 25, 100, 200, and 250 ml capacity.

6.12 Miscellaneous: tweezers, microspatula, rubber suction bulb, wood applicator stick, weighing paper, filter paper, and filter funnel with stand.

7. Reagents

All reagents used should be ACS reagent grade or better.

7.1 Bromine liquid, Br2. (Caution: corrosive liquid, causes severe burns, vapors extremely irritating and toxic. Wear gloves and handle only in a hood).

7.2 Sodium nitroferricyanide (sodium nitroprusside), Na2Fe(CN)5NO.2H2O.

7.3 Hydroxylamine hydrochloride, NH2OH.HCl

7.4 Sodium bicarbonate, NaHCO3

7.5 Hexane

7.6 Methanol

7.7 Distilled or deionized water

7.8 Complexing Reagent

7.8.1 Weigh 0.500 g sodium nitroferricyanide in a 50 ml beaker. Add 10 ml distilled water to the beaker and swirl to dissolve.

7.8.2 Weigh 0.500 g hydroxylamine hydrochloride and 1.000 g sodium bicarbonate. Transfer both materials to a mortar and carefully grind them together.

7.8.3 The next two steps must be performed in a hood. Add the ground mixture to the sodium nitroferricyanide solution. A bubbling reaction occurs for a few minutes. When the reaction is complete, add 0.10 ml bromine (see "caution" on Section 7.1). When effervescence stops, add approximately 10 ml distilled water and filter the solution. Rinse the beaker with 4 ml distilled water and filter. Transfer filtrate to a 25 ml volumetric flask and dilute with distilled water to the mark. This reagent should be kept refrigerated.

7.8.4 Dilute complexing reagent. Mix one part of complexing reagent with two parts water. Prepare this solution daily when needed.

7.9 Ethylene thiourea (2-imidazolidinethione). Usually this material is less than 99% pure and needs to be purified by recrystallization.

7.9.1 Recrystallization. In a 250 ml Erlenmeyer flask, weigh 3-5 g ethylene thiourea. Add 100 ml 1:1 methanol-water to dissolve the material. In a hood, heat the mixture to

boiling. Cool the flask at room temperature for five minutes. Add 5 ml hexane and shake the flask for 30 seconds. Cover the flask with a watch glass and leave undisturbed at room temperature for one hour. Filter the purified crystals, washing with 100 ml methanol-water mixture. Let the crystals air dry in the hood.

7.9.2 Caution: Ethylene thiourea has been found to be a teratogen and carcinogen in experimental animals. Extra care must be taken to avoid inhalation or skin contact with this material. Keep all material in a labeled bottle clearly identified as "potential human carcinogen," and place in a resealable thick-walled plastic bag, in locked storage.

7.10 Ethylene thiourea 1000 g/ml stock solution.

7.10.1 Weigh 0.250 g of recrystallized ethylene thiourea in a beaker. Add distilled water to dissolve the material. Transfer the solution into a 250 ml volumetric flask and dilute with distilled water to the mark.

8. Procedure

8.1 Cleaning of Equipment

8.1.1 Wash all glassware with detergent solution, rinse with tap water, distilled water, and dry in an oven.

8.2 Calibration of sampling pump. The personal sampling pump should be calibrated with a representative filter assembly in the line. A wet or dry test meter or a glass rotameter capable of measuring the flow rate to within $\pm$5% may be used for the calibration.

8.3 Collection and Shipping of Samples

8.3.1 Assemble the filters in the filter holder so that the air being sampled passes first through the PVC filter and then through the filter support. Remove the small plugs from the filter holder and connect the filter holder to the sampling pump by means of an adapter and a length of tubing.

8.3.2 Sample at least 100 liters of air. The optimum sample volume will depend on the type of workroom environment being sampled. Since high concentrations of particulate material may plug the filter, the flow rate should be

checked at least once every hour. On completion of sampling, reinsert the small plugs into the inlet and outlet of the filter holder. Record the temperature and pressure of the air being sampled.

8.3.3 With each group of samples prepare two blanks consisting of a filter holder with a representative filter that has been handled in the same manner as the sample filters, except that no air is drawn through them. The samples and the blank should be shipped promptly in a damage-proof container that allows no filter holder movement. Samples should be refrigerated as soon as possible.

8.4 Analysis of Samples

8.4.1 Extraction

1. Remove the top portion of the filter holder. Hold the bottom portion containing the filter and filter support over a piece of weighing paper to catch any particulate material that may fall out. Remove the small plug from the bottom portion of the filter holder and insert the applicator stick through the hole. Gently raise the filter support and the filter and grasp the unexposed edge with tweezers. Very carefully pick up the filter, insert it in a glass

vial, and push it gently to the bottom with the applicator stick. Add to the vial any particulate material remaining in the filter holder or collected on the weighing paper.

2. Pipet 7.0 ml of distilled water into the vial. This amount should completely cover the filter. Screw the cap on the vial.

3. Place vials in a 60C water bath (thermostatically controlled) for 45 minutes. The water bath level must be above the water level of the vial. Shake each vial every 5 minutes. The use of ultrasonic bath is not recommended because it breaks up the PVC filter.

4. Lift the filter with tweezers so it is above the water level in the vial and wash the filter eight times with 1 ml aliquots of water using a 1 ml pipette. Position the filter over the center of the vial so the rinsings can be collected in the vial, then discard the filter.

8.4.2 Complexation

This step should be performed at the same time for both standard and field samples so that the color formation

will start at the same time. This reduces color degradation discrepancies.

1. Pipet a 1.5 ml aliquot of the dilute complexing reagent into each vial.

2. Allow the vials to stand for at least 30 minutes before analysis to insure full color development. Shake the vials every 10 minutes.

8.4.3 Analysis

1. Transfer the solution to a clean 5-cm optical path length glass cell. Wipe off with a lens paper any droplets left on the cell windows.

2. Place the cell in the sample compartment and measure the absorbance at 590 nm. The reference sample contains 15 ml distilled water and 1.5 ml dilute complexing reagent in a 5-cm glass cell. Record the absorbance for each sample.

8.5 Determination of Extraction Efficiency

8.5.1 The extraction efficiency of ethylene thiourea may vary from laboratory to laboratory. The average percent recovery determined from 21 spiked filter samples was 98.7% with a 3% relative standard deviation in the 15-150 g/sample range.

8.5.2 Procedure

1. On a plastic test tube rack place eight PVC filters. Using an adjustable pipette, add to the center of each filter 0, 15, 30, 45, 60, 90, 120, and 150 microliters of the 1000 g/ml ethylene thiourea stock solution. This corresponds to concentrations of 0, 15, 30, 45, 60, 90, 120, and 150 micrograms per filter. Let filters air-dry overnight at room temperature. Place each spiked filter in a vial, and mark its concentration. Follow sections 8.4.1 parts 2 through 8.4.3 for preparation and analyses of the spiked samples.

2. The absorbance of each sample is converted to micrograms from the calibration curve (Section 10.1). Percent recovery is determined as follows:

$$\text{Percent recovery} = \frac{\text{micrograms recovered}}{\text{micrograms added}} \times 100$$

9. Calibration and Standardization

9.1 Ethylene thiourea 15 g/ml standard solution.

9.1.1 Pipet 3 ml aliquot of the 1000 g/ml stock solution into a 200 ml volumetric flask. Dilute with distilled water to the mark.

9.2 Preparation of Standard Samples

9.2.1 Prepare the standards by following Table I. Pipet aliquots of the 15 g/ml standard solution and distilled water in a marked vial.

TABLE I

Volume 15 g/ml std	Volume distilled water	Concentration
ml	ml	g/16.5 ml sample solution
0.00	15.00	0
1.00	14.00	15
2.00	13.00	30
3.00	12.00	45
4.00	11.00	60
6.00	9.00	90
8.00	7.00	120
10.00	5.00	150

Standards contain 1.5 ml of dilute complexing reagent. Total volume is 16.5 ml/vial. Follow section 8.4.2 and 8.4.3 to complex and analyze the standards.

9.2.2 Standards must be prepared and analyzed in conjunction with field samples. Analyses of standards should be performed before and after field samples are analyzed.

9.2.3 Prepare a calibration curve by plotting absorbance vs concentration of standards in g/16.5 ml sample.

10. Calculations

10.1 The concentration of ethylene thiourea in field samples can be determined graphically from the plot of absorbance vs concentration.

10.2 The concentration of ethylene thiourea in air may be expressed in mg/m3 .

10.3 For personal pumps with rotameters only, the following correction for air volumes sampled should be made:

$$\text{Corrected Volume} = f \times t \sqrt{\frac{P1}{P2} \times \frac{T2}{T1}}$$

where:

f = sample flow rate

t = sampling time

P1= pressure during calibration of sampling pump (mm Hg)

P2= pressure of air sampled (mm Hg)

T1= temperature during calibration of sampling pump (K)

T2= temperature of air sampled (K)

11. References

11.1 Grote WI: "A New Color Reaction for Soluble Organic Sulfur Compounds," J. Biol. Chem., 93, 25, 1931

11.2 Danowski TS: "Measurement of Thiourea in Ultrafiltrate of Serum," J. Biol. Chem., 152, 201, 1944

11.3 Association of Official Agricultural Chemists, 31, 100, 1948