

DETERMINATION OF EBDC IN CROPS, MEAT AND MILK

000382

ETU 89 0

DETERMINATION OF ETHYLENE BIS DITHIOCARBAMATES (EBDC'S)
IN CROPS AND PROCESSED COMMODITIES

METHOD NO: ETU-89AM-001

Gary L. Westberg
MORSE LABORATORIES, INC.
Sacramento, CA 95825

DATE: 06/07/89

PRINCIPLE:

EBDC's present in the sample are converted to CS₂ during reaction with HCl/Stannous chloride reagent at 100°C in a sealed reaction flask. An aliquot of the headspace is injected into a gas chromatograph where the sample responses are compared to an EBDC standard similarly prepared and injected.

REAGENTS:

- Maneb - analytical grade, Pennwalt Corporation,
Philadelphia, PA 19102
- Mancozeb - analytical grade, Rohm and Haas Company,
Philadelphia, PA 19105
- E. I. DuPont de Nemours & Co., Inc.
Wilmington, DE 19898
- Metiram - analytical grade, BASF Corporation,
Parsippany, NJ 07054
- GLC Column Packing - PT 28% Alltech 223 + 4% KOH on 80/100 Gas
Chrom R, Alltech Associates, Inc.,
Deerfield, IL 60015
- EDTA (tetrasodium) - over 99% purity, EM Science,
Cherry Hill, NJ 08034.
- EDTA Solution - 10% (w/v) in boiled deionized water.
- Hydrochloric Acid - concentrated, J.T. Baker Chemical Co.,
Phillipsbury, N.J. 08665
- Stannous Chloride - analytical grade, Spectrum Chemical Mfg.,
Gardena, CA 90248

Carbon disulfide - reagent grade, J.T. Baker Chemical Co.
Phillipsbury, N.J. 08665

Methanol - nanograde, J.T. Baker Chemical Co.
Phillipsbury, N.J. 08665

Hexane - nanograde, J.T. Baker Chemical Co.
Phillipsbury, N.J. 08665

HCl/Stannous chloride reagent - 8N:3%; weigh 3.6 g $\text{SnCl}_2 \cdot (\text{H}_2\text{O})_2$
into a 100 mL volumetric flask.
Add 64 mL concentrated HCl. QS
to 100 mL with boiled deionized
water.

APPARATUS:

Reaction flasks - 160 mL equipped for crimp sealing with
teflon lined septums, Pierce Chemical Co.,
Rockford, IL 61105, cat. #12995

Crimp seals
with teflon
lined septums - Supelco, Inc., Bellefonte, PA 16823,
cat. #3-3118

Crimper - Wheaton Instruments, Millville, NJ,
part #224303

Water Bath

Gas Chromatograph - Microtek MT-220 or equivalent with a flame
photometric detector in the sulfur mode.

Gas Chromatographic
Column - 6' x 1/4" o.d. x 4 mm i.d. glass column
packed with PT 28% Alltech 223 + 4% KOH on
80/100 Gas Chrom R.

Gas tight
syringes - 10, 50, 100, 250, 500, 1000, 2500 uL,
Hamilton Co., Reno, NV

Waring Blender

Hobart meat grinder
(or equivalent)

Reitz disintegrator
grinder, Wiley mill,
or equivalent

"Airpettor" Adjustable
pipettes

- American SMI "Airpettor", Scientific
Products, Sunnyvale, CA 94089

Pipettes: 50-200 uL volume range cat. #P5086-2
200-1000 uL volume range cat. #P5086-3
Tips: 2-200 uL cat. #P5059-301
100-1000 uL cat. #P5059-801

STANDARD PREPARATION:

1. Carbon disulfide (CS₂): 100 uL CS₂ is dissolved in 100 mL hexane. 4 uL of the 0.1% solution is placed in a reaction flask, which is immediately sealed and heated to 100°C to allow all hexane and CS₂ to equilibrate in headspace. This standard is used to locate the gas chromatographic CS₂ response.
2. EBDC: Correcting for purity, weigh 10.0 mg active ingredient of EBDC analytical standard into a 250 mL beaker. Using a 100 mL volumetric pipet, deliver 100 mL boiled deionized water (cooled) into the beaker containing the EBDC standard. With a Power Pulse homogenizer (or equivalent, i.e. Polytron homogenizer), homogenize the EBDC suspension for 10 minutes. (Note: the resulting suspension must be homogeneous and devoid of heavy particles). While the suspension is being homogenized, remove 10.0 mL with a graduated pipet and dilute to 100 mL with boiled deionized water. (Note: 10 mL aliquot must be withdrawn quickly and accurately on first attempt. If the initial withdrawal overshoots or undershoots 10 mL mark, the withdrawal procedure must be reinitiated.) This makes a 10 ug/mL solution. Remove 1.35 mL from the 10 ug/mL suspension while it is being homogenized by use of the "Airpettor" and add it to a 160 mL reaction flask. (Note: Take two 675 uL aliquots in place of one 1.35 mL aliquot.) Add 8.65 mL 10% EDTA solution and 15 mL HCl/Stannous chloride reagent to the reaction flask and immediately crimp seal. React contents of flask in the same fashion as samples, as discussed later. Resulting concentration of prepared EBDC standard for gas chromatography is 0.1 ug/mL headspace. The least stable EBDC suspension standard is analytically stable (<3% degradation) for 30 minutes. All manipulations with suspension standards must be completed within this time frame.

PROCEDURES:

Sample Preparation:

Wet crops are ground in the Hobart grinder while frozen with dry ice. Dry crops are ground while frozen through a Reitz disintegrator grinder with dry ice. Individual crops that require further grinding are ground with dry ice in a Waring blender at the discretion of the analyst and with proper documentation.

Extraction:

Place 4.0 g of frozen, ground sample in 160 mL reaction flask. Add 10% EDTA solution to make a total volume of 10 mL (sample plus 10% EDTA solution). This volume must be determined for each crop prior to analysis, based on the volume of 4 g ground sample (see discussion). Add 15.0 mL HCl/Stannous Chloride (8N/3%) reagent. Immediately seal. Place flask in boiling waterbath for 2 hours, hand shaking flasks approximately every 5 minutes for the first 30 minutes, then every 30 minutes for the remaining 1 1/2 hours. After reaction, maintain sample at 100°C in the waterbath during GLC analysis. Some crops, such as corn forage, may require reaction for more than 2 hours or a reduction in sample size. This modification is only needed on matrixes where the procedure produces apparent poor recoveries at the standard reaction time of two hours and the standard sample size of 4.0 g.

Sample Fortification:

Make a 10 ug/mL standard as above under standard preparation. While this suspension is being homogenized, remove 10.0 mL and dilute to 100 mL with deionized water. This makes a 1.0 ug/mL solution. All fortifications of samples are done from these solutions as they are being homogenized. Use only pipettes or "Airpettors" to add the fortification solutions to the sample. DO NOT USE MICROLITER SYRINGES.

Place 4.0 g frozen ground sample into a 160 mL reaction flask. Fortify the samples at the correct level by adding the appropriate volume from the 1.0 ug/mL or 10 ug/mL fortification solutions. Add 10% EDTA solution to make a total volume of 10 mL (sample plus fortification solution plus 10% EDTA solution). Add 15.0 mL HCl/Stannous Chloride (8N/3%) reagent. Immediately seal. Treat as samples from this point on, heating the fortification at 100°C for the same time as the samples.

Gas Chromatographic Analysis:

Equilibrate the gas chromatograph as follows: Injector Temperature - 240°C, Detector Temperature - 200°C, Column Temperature - 130°C, Nitrogen Carrier Gas Flow - 40 mL/min.

Condition column overnight at 200°C with 30 mL/min carrier gas flow. Prior to analysis, make several injections (10-100 uL) of EBDC standard (0.1 ug/mL headspace) to sensitize the column for CS₂. Under these gas chromatographic conditions, the retention time for EBDC as CS₂ is 2-3 minutes.

Prepare a 4 point standard curve by chromatographing 0.4 ng through 2.5 ng of appropriate EBDC standard. (Note: 2.5 ng may produce an off scale response on some FPD detectors due to their inherent differences in logarithmic response. In such cases, inject an amount of standard which produces a 90% FSD as the high point of the curve. In any case, 0.4 ng, representing a 0.0135 ppm response when 1000 uL of sample is injected, must be included as the lowest point on the standard curve and should produce a response peak height of at least 8 mm. Identification of the peak produced by the EBDC standard as CS₂ is achieved by demonstrating its retention time to be identical to that of the peak produced by an injection of CS₂ standard.

Inject 2 - 1000 uL of airspace from the samples into the gas chromatograph using air-tight syringes. Compare sample responses to those produced in the standard curve.

Calculation is made by use of the following equation:

$$\text{ppm} = \frac{\text{ng EBDC}}{\text{mg sample injected}}$$

where: ng EBDC is derived from standard curve

$$\text{mg sample injected} = \frac{4.0 \text{ g} \times \text{uL injected}}{\text{headspace volume of sample-containing reaction flask}}$$

where: headspace volume of sample-containing reaction flask = 135 mL.

NOTE: Standard curve is prepared by plotting ng injected vs peak height on log/log graph paper.

DISCUSSION:

The gas chromatographic method described herein has a detection limit of 0.02 ppm for the EBDC's maneb, metiram and mancozeb.

In order to eliminate a possibility of CS₂ contamination, reaction vials were rinsed in methanol and baked at 100-110°C for 45 minutes. Teflon lined septums were rinsed with methanol and air dried. The use of rubber or plastic utensils was avoided.

The volume of 4.0 g of matrix is determined by weighing 4.0 g of sample into a 25 mL graduated cylinder and adding 10 mL water. The total volume minus 10 mL is the volume of the 4 g sample. (Note: Better results may be achieved with some matrices if 20 mL of water is used in place of 10 mL.)

Standard curves are prepared by injection of variable volumes of a single standard preparation at a concentration of 0.1 ug/mL headspace.

Samples must be kept frozen at all times until addition of extraction/reaction reagents. This includes weighing and fortification (preparation of spikes) processes. Keep samples on dry ice before and after weighing and during fortification.

Samples must be reacted immediately following addition of reaction reagents. Once reacted, the samples (now containing in the form of CS₂ any EBDC that may have been present) may be stored at room temperature overnight. Simply reheat the samples the following day at 100°C for approximately 30 minutes with shaking.

REFERENCES:

- (1) JAOAC, Vol. 52, No. 6, page 1226 (1969)
- (2) "Determination of Maneb in Crops", Morse Laboratories, Inc., Sacramento, CA 95825, Method No. MTF-88AM-005, 6/15/87

DETERMINATION OF ETHYLENE BIS DITHIOCARBAMATES (EBDC'S)
IN MEAT

METHOD NO: ETU-89AM-002

Gary L. Westberg
MORSE LABORATORIES, INC.
Sacramento, CA 95825

DATE: 08/25/89

PRINCIPLE:

EBDC's present in the sample are converted to CS₂ during reaction with HCl/Stannous chloride reagent at 100°C in a sealed reaction flask. An aliquot of the headspace is injected into a gas chromatograph where the sample responses are compared to an EBDC standard similarly prepared and injected.

REAGENTS:

Maneb - analytical grade, Pennwalt Corporation,
Philadelphia, PA 19102

Mancozeb - analytical grade, Rohm and Haas Company,
Philadelphia, PA 19105

E. I. DuPont de Nemours & Co., Inc.
Wilmington, DE 19898

Metiram - analytical grade, BASF Corporation,
Parsippany, NJ 07054

GLC Column Packing - PT 28% Alltech 223 + 4% KOH on 80/100 Gas
Chrom R or 28% Pennwalt 223 + 4% KOH on
80/100 Gas Chrom R, Alltech Associates,
Inc., Deerfield, IL 60015

EDTA (tetrasodium) - over 99% purity, EM Science,
Cherry Hill, NJ 08034.

EDTA Solution - 10% (w/v) in boiled deionized water.

Hydrochloric Acid - concentrated, J.T. Baker Chemical Co.,
Phillipsbury, N.J. 08665

Stannous Chloride - analytical grade, Spectrum Chemical Mfg.,
Gardena, CA 90248

MORSE LABORATORIES, INC.
ANALYTICAL METHOD NO: ETU-89AM-002

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Carbon disulfide - reagent grade, J.T. Baker Chemical Co.
Phillipsbury, N.J. 08665

Methanol - nanograde, J.T. Baker Chemical Co.
Phillipsbury, N.J. 08665

Hexane - nanograde, J.T. Baker Chemical Co.
Phillipsbury, N.J. 08665

HCl/Stannous chloride reagent - 8N:3%; weigh 3.6 g $\text{SnCl}_2 \cdot (\text{H}_2\text{O})_2$
into a 100 mL volumetric flask.
Add 64 mL concentrated HCl. QS
to 100 mL with boiled deionized
water.

APPARATUS:

Reaction flasks - 160 mL equipped for crimp sealing with
teflon lined septums, Pierce Chemical Co.,
Rockford, IL 61105, cat. #12995

Crimp seals
with teflon lined
silicone septums - Pierce Chemical Co., Rockford, IL 61105,
cat. #12720 (septums) and #13214 (caps).

Crimper - Wheaton Instruments, Millville, NJ,
part #224303

Water Bath

Gas Chromatograph - Microtek MT-220 or equivalent with a flame
photometric detector in the sulfur mode.

Gas Chromatographic
Column - 6' x 1/4" o.d. x 4 mm i.d. glass column
packed with PT 28% Alltech 223 + 4% KOH on
80/100 Gas Chrom R or 28% Pennwalt 223+ 4%
KOH on 80/100 Gas Chrom R.

Gas tight
syringes - 10, 50, 100, 250, 500, 1000, 2500 uL,
Hamilton Co., Reno, NV

Hobart meat grinder
(or equivalent)

"Airpettor"
Adjustable
pipettes

- American SMI "Airpettor", Scientific
Products, Sunnyvale, CA 94089 or
equivalent.

Pipettes: 50-200 uL volume range
200-1000 uL volume range
Tips: 40-200 uL
100-1000 uL

STANDARD PREPARATION:

1. Carbon disulfide (CS₂): 100 uL CS₂ is dissolved in 100 mL hexane. 4 uL of the 0.1% solution is placed in a reaction flask, which is immediately sealed and heated to 100°C to allow all hexane and CS₂ to equilibrate in headspace. This standard is used to locate the gas chromatographic CS₂ response.
2. EBDC: Correcting for purity, weigh 10.0 mg active ingredient of EBDC analytical standard into a 250 mL beaker. Using a 100 mL volumetric pipet, deliver 100 mL boiled deionized water (cooled) into the beaker containing the EBDC standard. With a Power Pulse homogenizer (or equivalent, i.e. Polytron homogenizer), homogenize the EBDC suspension for 10 minutes. (Note: the resulting suspension must be homogeneous and devoid of heavy particles). While the suspension is being homogenized, remove 10.0 mL with a graduated pipet and dilute to 100 mL with boiled deionized water. (Note: 10 mL aliquot must be withdrawn quickly and accurately on first attempt. If the initial withdrawal overshoots or undershoots 10 mL mark, the withdrawal procedure must be reinitiated.) This makes a 10 ug/mL solution. Remove 0.27 mL (or 270 uL) from the 10 ug/mL suspension while it is being homogenized by use of the "Airpettor" and add it to a 160 mL reaction flask. Add 9.73 mL 10% EDTA solution and 15 mL HCl/Stannous chloride reagent to the reaction flask and immediately crimp seal. React contents of flask in the same fashion as samples, as discussed later. Resulting concentration of prepared EBDC standard for gas chromatography is 0.02 ug/mL headspace. The least stable EBDC suspension standard is analytically stable (<3% degradation) for 30 minutes. All manipulations with suspension standards must be completed within this time frame.

PROCEDURES:

Sample Preparation:

The meat is ground in the Hobart grinder while frozen with dry ice.

Extraction:

Place 10.0 g of frozen, ground sample in 160 mL reaction flask. Add 10.0 mL of 10% EDTA solution and 15.0 mL HCl/Stannous Chloride (8N/3%) reagent. Immediately seal. Place flask in boiling waterbath for 2 hours, hand shaking flasks approximately every 5 minutes for the first 30 minutes, then every 30 minutes for the remaining 1 1/2 hours. After reaction, maintain sample at 100°C in the waterbath during GLC analysis.

Sample Fortification:

Make a 10 ug/mL standard as above under standard preparation. While this suspension is being homogenized, remove 10.0 mL and dilute to 100 mL with deionized water. This makes a 1.0 ug/mL solution. All fortifications of samples are done from these solutions as they are being homogenized. Use only pipettes or "Airpettors" to add the fortification solutions to the sample. DO NOT USE MICROLITER SYRINGES.

Place 10.0 g frozen ground sample into a 160 mL reaction flask. Fortify the samples at the correct level by adding the appropriate volume from the 5.0 ug/mL, 1.0 ug/mL or 10 ug/mL fortification solutions. Add 10.0 mL 10% EDTA solution and 15.0 mL HCl/Stannous Chloride (8N/3%) reagent. Immediately seal. Treat as samples from this point on, heating the fortification at 100°C for the same time as the samples.

Gas Chromatographic Analysis:

Equilibrate the gas chromatograph as follows: Injector Temperature - 240°C, Detector Temperature - 160°C, Column Temperature - 115°C, Nitrogen Carrier Gas Flow - 35 mL/min. Condition column overnight at 200°C with 30 mL/min carrier gas flow. Prior to analysis, make several injections (10-100 uL) of EBDC standard (0.02 ug/mL headspace) to sensitize the column for CS₂. Under these gas chromatographic conditions, the retention time for EBDC as CS₂ is 6 minutes.

Prepare a 4 point standard curve by chromatographing 0.12 ng through 1.0 ng of appropriate EBDC standard. (Note: 1.0 ng may produce an off scale response on some FPD detectors due to their inherent differences in logarithmic response. In such cases, inject an amount of standard which produces a 90% FSD as the high point of the curve. In any case, 0.12 ng, representing a 1.4 ppb

response when 1000 uL of sample is injected, must be included as the lowest point on the standard curve and should produce a response peak height of at least 8 mm. Identification of the peak produced by the EBDC standard as CS₂ is achieved by demonstrating its retention time to be identical to that of the peak produced by an injection of CS₂ standard.

Inject 2 - 1000 uL of airspace from the samples into the gas chromatograph using air-tight syringes. Compare sample responses to those produced in the standard curve.

Calculation is made by use of the following equation:

$$\text{ppb} = \frac{\text{ng EBDC}}{\text{mg sample injected}} \times 1000$$

where: ng EBDC is derived from standard curve

$$\text{mg sample injected} = \frac{10.0 \text{ g} \times \text{uL injected}}{\text{headspace volume of sample-containing reaction flask}}$$

where: headspace volume of sample-containing reaction flask = 125 mL.

NOTE: Standard curve is prepared by plotting ng injected vs peak height on log/log graph paper.

DISCUSSION:

The gas chromatographic method described herein has a detection limit of 2.0 ppb for the EBDC's maneb, metiram and mancozeb.

In order to eliminate a possibility of CS₂ contamination, reaction vials were rinsed in methanol and baked at 100-110°C for 45 minutes. Teflon lined septums were rinsed with methanol and air dried. The use of rubber or plastic utensils was avoided.

Standard curves are prepared by injection of variable volumes of a single standard preparation at a concentration of 0.02 ug/mL headspace.

Samples must be kept frozen at all times until addition of extraction/reaction reagents. This includes weighing and fortification (preparation of spikes) processes. Keep samples on dry ice before and after weighing and during fortification.

Samples must be reacted immediately following addition of reaction reagents. Once reacted, the samples (now containing in the form of CS₂ any EBDC that may have been present) may be stored at room temperature overnight. Simply reheat the samples the following day at 100°C for approximately 30 minutes with shaking.

REFERENCES:

- (1) JAOAC, Vol. 52, No. 6, page 1226 (1969)
- (2) "Determination of Maneb in Crops", Morse Laboratories, Inc., Sacramento, CA 95825, Method No. MTF-88AM-005, 6/15/87

DETERMINATION OF ETHYLENE BIS DITHIOCARBAMATES (EBDC'S)
IN MILK

METHOD NO: ETU-89AM-003

Gary L. Westberg
MORSE LABORATORIES, INC.
Sacramento, CA 95825

DATE: 08/25/89

PRINCIPLE:

EBDC's present in the sample are converted to CS₂ during reaction with HCl/Stannous chloride reagent at 100°C in a sealed reaction flask. An aliquot of the headspace is injected into a gas chromatograph where the sample responses are compared to an EBDC standard similarly prepared and injected.

REAGENTS:

- Maneb - analytical grade, Pennwalt Corporation,
Philadelphia, PA 19102
- Mancozeb - analytical grade, Rohm and Haas Company,
Philadelphia, PA 19105
- E. I. DuPont de Nemours & Co., Inc.
Wilmington, DE 19898
- Metiram - analytical grade, BASF Corporation,
Parsippany, NJ 07054
- GLC Column Packing - PT 28% Alltech 223 + 4% KOH on 80/100 Gas
Chrom R or 28% Pennwalt 223 + 4% KOH on
80/100 Gas Chrom R, Alltech Associates,
Inc., Deerfield, IL 60015
- EDTA (tetrasodium) - over 99% purity, EM Science,
Cherry Hill, NJ 08034.
- EDTA Solution - 10% (w/v) in boiled deionized water.
- Hydrochloric Acid - concentrated, J.T. Baker Chemical Co.,
Phillipsbury, N.J. 08665
- Stannous Chloride - analytical grade, Spectrum Chemical Mfg.,
Gardena, CA 90248

Carbon disulfide - reagent grade, J.T. Baker Chemical Co.
Phillipsbury, N.J. 08665

Methanol - nanograde, J.T. Baker Chemical Co.
Phillipsbury, N.J. 08665

Hexane - nanograde, J.T. Baker Chemical Co.
Phillipsbury, N.J. 08665

HCl/Stannous chloride reagent - 8N:3%; weigh 3.6 g $\text{SnCl}_2 \cdot (\text{H}_2\text{O})_2$
into a 100 mL volumetric flask.
Add 64 mL concentrated HCl. QS
to 100 mL with boiled deionized
water.

APPARATUS:

- Reaction flasks - 160 mL equipped for crimp sealing with
teflon lined septums, Pierce Chemical Co.,
Rockford, IL 61105, cat. #12995
- Crimp seals
with teflon lined
silicone septums - Pierce Chemical Co., Rockford, IL 61105,
cat. #12720 (septums) and #13214 (caps).
- Crimper - Wheaton Instruments, Millville, NJ,
part #224303
- Water Bath
- Gas Chromatograph - Microtek MT-220 or equivalent with a flame
photometric detector in the sulfur mode.
- Gas Chromatographic
Column - 6' x 1/4" o.d. x 4 mm i.d. glass column
packed with PT 28% Al1tech 223 + 4% KOH on
80/100 Gas Chrom R or 28% Pennwalt 223 + 4%
KOH on 80/100 Gas Chrom R.
- Gas tight
syringes - 10, 50, 100, 250, 500, 1000, 2500 uL,
Hamilton Co., Reno, NV

"Airpettor"
Adjustable
pipettes

- American SMI "Airpettor", Scientific
Products, Sunnyvale, CA 94089 or
equivalent.

Pipettes: 50-200 uL volume range
200-1000 uL volume range
Tips: 40-200 uL
100-1000 uL

STANDARD PREPARATION:

1. Carbon disulfide (CS₂): 100 uL CS₂ is dissolved in 100 mL hexane. 4 uL of the 0.1% solution is placed in a reaction flask, which is immediately sealed and heated to 100°C to allow all hexane and CS₂ to equilibrate in headspace. This standard is used to locate the gas chromatographic CS₂ response.
2. EBDC: Correcting for purity, weigh 10.0 mg active ingredient of EBDC analytical standard into a 250 mL beaker. Using a 100 mL volumetric pipet, deliver 100 mL boiled deionized water (cooled) into the beaker containing the EBDC standard. With a Power Pulse homogenizer (or equivalent, i.e. Polytron homogenizer), homogenize the EBDC suspension for 10 minutes. (Note: the resulting suspension must be homogeneous and devoid of heavy particles). While the suspension is being homogenized, remove 10.0 mL with a graduated pipet and dilute to 100 mL with boiled deionized water. (Note: 10 mL aliquot must be withdrawn quickly and accurately on first attempt. If the initial withdrawal overshoots or undershoots 10 mL mark, the withdrawal procedure must be reinitiated.) This makes a 10 ug/mL solution. Remove 0.27 mL (or 270 uL) from the 10 ug/mL suspension while it is being homogenized by use of the "Airpettor" and add it to a 160 mL reaction flask. Add 9.73 mL 10% EDTA solution and 15 mL HCl/Stannous chloride reagent to the reaction flask and immediately crimp seal. React contents of flask in the same fashion as samples, as discussed later. Resulting concentration of prepared EBDC standard for gas chromatography is 0.02 ug/mL headspace. The least stable EBDC suspension standard is analytically stable (<3% degradation) for 30 minutes. All manipulations with suspension standards must be completed within this time frame.

PROCEDURES:

Sample Preparation:

The frozen sample is quick-thawed to a slush using a cold water bath.

Extraction:

Place 20.0 g of frozen sample in 160 mL reaction flask. Add 10.0 mL of 10% EDTA solution and 15.0 mL HCl/Stannous Chloride (8N/3%) reagent. Immediately seal. Place flask in boiling waterbath for 2 hours, hand shaking flasks approximately every 5 minutes for the first 30 minutes, then every 30 minutes for the remaining 1 1/2 hours. After reaction, maintain sample at 100°C in the waterbath during GLC analysis.

Sample Fortification:

Make a 10 ug/mL standard as above under standard preparation. While this suspension is being homogenized, remove 10.0 mL and dilute to 100 mL with deionized water. This makes a 1.0 ug/mL solution. All fortifications of samples are done from these solutions as they are being homogenized. Use only pipettes or "Airpettors" to add the fortification solutions to the sample. DO NOT USE MICROLITER SYRINGES.

Place 20.0 g frozen ground sample into a 160 mL reaction flask. Fortify the samples at the correct level by adding the appropriate volume from the 1.0 ug/mL or 10 ug/mL fortification solutions. Add 10.0 mL 10% EDTA solution and 15.0 mL HCl/Stannous Chloride (8N/3%) reagent. Immediately seal. Treat as samples from this point on, heating the fortification at 100°C for the same time as the samples.

Gas Chromatographic Analysis:

Equilibrate the gas chromatograph as follows: Injector Temperature - 240°C, Detector Temperature - 160°C, Column Temperature - 115°C, Nitrogen Carrier Gas Flow - 35 mL/min. Condition column overnight at 200°C with 30 mL/min carrier gas flow. Prior to analysis, make several injections (10-100 uL) of EBDC standard (0.02 ug/mL headspace) to sensitize the column for CS₂. Under these gas chromatographic conditions, the retention time for EBDC as CS₂ is 6 minutes.

Prepare a 4 point standard curve by chromatographing 0.12 ng through 1.0 ng of appropriate EBDC standard. (Note: 1.0 ng may produce an off scale response on some FPD detectors due to their inherent differences in logarithmic response. In such cases, inject an amount of standard which produces a 90% FSD as the high point of the curve. In any case, 0.12 ng, representing a 1.4 ppb

response when 500 uL of sample is injected, must be included as the lowest point on the standard curve and should produce a response peak height of at least 8 mm. Identification of the peak produced by the EBDC standard as CS₂ is achieved by demonstrating its retention time to be identical to that of the peak produced by an injection of CS₂ standard.

Inject 2 - 500 uL of airspace from the samples into the gas chromatograph using air-tight syringes. Compare sample responses to those produced in the standard curve.

Calculation is made by use of the following equation:

$$\text{ppb} = \frac{\text{ng EBDC}}{\text{mg sample injected}} \times 1000$$

where: ng EBDC is derived from standard curve

$$\text{mg sample injected} = \frac{20.0 \text{ g} \times \text{uL injected}}{\text{headspace volume of sample-containing reaction flask}}$$

where: headspace volume of sample-containing reaction flask = 115 mL.

NOTE: Standard curve is prepared by plotting ng injected vs peak height on log/log graph paper.

DISCUSSION:

The gas chromatographic method described herein has a detection limit of 2.0 ppb for the EBDC's maneb, metiram and mancozeb.

In order to eliminate a possibility of CS₂ contamination, reaction vials were rinsed in methanol and baked at 100-110°C for 45 minutes. Teflon lined septums were rinsed with methanol and air dried. The use of rubber or plastic utensils was avoided.

Standard curves are prepared by injection of variable volumes of a single standard preparation at a concentration of 0.02 ug/mL headspace.

Samples must be kept frozen at all times until addition of extraction/reaction reagents. This includes weighing and fortification (preparation of spikes) processes. Keep samples on dry ice before and after weighing and during fortification.

Samples must be reacted immediately following addition of reaction reagents. Once reacted, the samples (now containing in the form of CS₂ any EBDC that may have been present) may be stored at room temperature overnight. Simply reheat the samples the following day at 100°C for approximately 30 minutes with shaking.

REFERENCES:

- (1) JAOAC, Vol. 52, No. 6, page 1226 (1969)
- (2) "Determination of Maneb in Crops", Morse Laboratories, Inc., Sacramento, CA 95825, Method No. MTF-88AM-005, 6/15/87

Alternate Analytical Reagents, Apparatus and GC Conditions
Used In the First Quarter of the EBDC/ETU National Food Survey

Methods For Determination Of Ethylene Bis Dithiocarbamates
(EBDC's) In Crops And Processed Commodities, Meat and Milk:
Method Numbers ETU-89AM-001 (6/7/89); ETU-89AM-002 (8/25/89);
ETU-AM-003 (8/25/89)

In some cases, alternate reagents, apparatus and GC
conditions equivalent to those specified in Methods ETU-89AM-001,
-002, and -003 have been substituted. These substitutions are
specified below according to laboratory.

Craven Laboratories

REAGENTS

Water: Boiled MilliQ water
MilliQ water purification system,
Millipore Corporation
Milford, MA 01757

EDTA (tetrasodium): Over 99% purity, Baker Analyzed,
no. L693-07
Baker Chemical Co, Phillipsbury, NJ
08665

Stannous Chloride: Analytical grade, Baker Analyzed
no. 3980-05

Carbon Disulfide: Reagent grade, Fisher no. C184-500
Fisher Scientific, Fairlawn, NJ 07410

APPARATUS

Gas Chromatograph: Microtek MT 2200 Gas Chromatograph with
Flame Photometric Detector in the Sulfur
Mode
Tracor, Inc., 6500 Tracor Lane, Austin,
TX 78721

Linear Recorder Model 555
Linear Instrument Corporation
2325 Robb Drive, Reno, NV 89523

Gas chromatographic
Colum: 6' x 4 mm packed with 28% Pennwalt 223 +
4% KOH on 80/100 Gas Chrom R

Gas Chromatographic
Column for Initial
Confirmatory Test: 6' x 4 mm packed with Chromosorb 108
80/100 mesh

GC CONDITIONS (typical)

Injector Temperature: 225°C
Detector Temperature: 205°C
Column Temperature: 115°C
Nitrogen Carrier
Gas Flow: 45 ml/min
Chart Speed: 1 cm/min

EN-CAS Analytical Laboratories

REAGENTS

Water: Boiled MilliQ water
MilliQ water purification system,
Whatman, Whatman International, Ltd.,
Maidstone, England

EDTA (tetrasodium): 98% purity, Mallinckrodt, Paris,
Kentucky 40361

Hydrochloric Acid: Fisher Scientific, Fairlawn, NJ 07410

Stannous Chloride: Certified A.C.S., Fisher Scientific

Carbon Disulfide: Reagent grade, Fisher Scientific

Methanol: Pesticide, Fisher Scientific.

APPARATUS

Waring Blender: Not used

Reitz Disintegrator: Hobart meat grinder used

Crimp seals with silicone/teflon lined septums: Seals, no. 13214; Teflon/Silicon Discs, no. 12720
Pierce, Rockford, Illinois 61105

Gas Chromatograph: Tracor 540 with Flame Photometric Detector in Sulfur Mode.
Tracor, Inc., 6500 Tracor Lane, Austin, Texas 78721

Gas Tight Syringes: 25,100,1000 ul
Hamilton Co., Reno, Nevada

Gas Chromatographic Column (Corn): 6' x 1/4" o.d. x 4 mm i.d. packed with PT 28% Alltech 223 + 4% KOH on 80/100 Gas Chrom R, Supelco, Inc., Bellefonte, PA 16823

Gas Chromatographic Column (Dry Beans): 6' x 1/4" o.d. x 4mm i.d. packed with chromosorb 108, mesh 80/100 (same column used for initial confirmation at the other laboratories)

GC CONDITIONS (typical)

Injector Temperature: 210°C

Detector Temperature: 200°C

Column Temperature: 130°C

Helium Gas Carrier Flow: 40 ml/min

Chart Speed: 1.0 cm/min

McKenzie LaboratoriesREAGENTS:

Water: Deionized tap water, unless specified otherwise

Standard solutions of test materials were made with HPLC grade water prior to 10/27/89 and with deionized water after 10/27/89

EDTA: Over 99%, Mallinckrodt
Paris, Kentucky 40361

EDTA Solution: 10% (v/v) in boiled deionized water

Hydrochloric Acid: Concentrated, EM Science, P.O. Box 70,
480 Democrat Rd., Gibbstown, NJ 08027

Stannous Chloride: Analytical grade, Mallinckrodt

Methanol: B & J, Baxter Healthcare Corp,
Muskegon, MI 49442

APPARATUS

Gas tight syringes: 10, 50, 100, 250, 500, 1000, 2500 ul,
Hamilton Co., Reno, NV

Monoject 3cc lock syringe,
Sherwood Medical, St. Louis, MO 63101

Gas Chromatograph: Microtek MT 220 Gas Chromatograph with
Flame Photometric Detector in the Sulfur
Mode.
Tracor, Inc., 6500 Tracor Lane, Austin,
TX 78721

Tracor 540 Gas Chromatograph with Flame
Photometric Detector in the Sulfur Mode.
Tracor, Inc.

Gas Chromatographic
Column:

240 cm x 6 mm o.d. x 2 mm i.d. packed
with 28% Alltech 223 + 4% KOH on 80/100
Gas Chrom R

Gas Chromatographic
Column for Initial
Confirmatory Test:

180 cm x 6 mm o.d. x 4 mm i.d. packed
with Chromosorb 108 80/100 mesh

GC CONDITIONS (typical)

Microtek 220

Injector Temperature: 215°C
 Detector Temperature: 95°C
 Column Temperature: 235°C
 Nitrogen Carrier
 Gas Flow: 15 ml/min

Tracor 540

Injector Temperature: 200°C
 Detector Temperature: 80°C
 Column Temperature: 215°C
 Nitrogen Carrier
 Gas Flow: 0.4 ml/min

Microtek 220 (initial confirmatory test)

Injector Temperature: 260°C
 Detector Temperature: 255°C
 Column Temperature: 140°C
 Nitrogen Carrier
 Gas Flow: 35 ml/min
 Recorder Speed: 0.25 in/min

Morse LaboratoriesREAGENTS

Water: Boiled deionized water

EDTA (tetrasodium): Mallinckrodt, Paris, Kentucky, 40361

APPARATUS

Crimp seals with
silicone/teflon lined
septums:

Seals, no. 13214; Teflon/Silicon Discs,
no. 12720
Pierce, Rockford, Illinois 61105

Gas Chromatograph:

Microtek 220 Gas Chromatograph equipped
with a Meloy Flame Photometric detector
in the sulfur mode; Model MT-220 FPD-S.
Tracor, Inc., 6500 Tracor Lane,
Austin, TX 78721

Gas Chromatographic
Column:

6' x 1/4" o.d. x 5/32" i.d. packed with
28% Pennwalt 223 + 4% KOH on 80/100 Gas
Chrom R.

Gas Chromatographic
Column for Initial
Confirmatory Test:

6' x 1/4" o.d. x 5/32" i.d. packed with
Chromosorb 108 80/100 mesh.

GC CONDITIONS (typical)

Injector Temperature:	210°C	
Detector Temperature:	160°C	
Column Temperature:	115°C	(meat, milk, tomato, tomato paste, tomato juice, tomato puree)
	125°C	(ketchup)
	135°C	(potato, frozen potato fries)
	160°C	(initial confirmatory test)

Nitrogen Carrier

Gas Flow: 20 ml/min (tomato paste)
25 ml/min (tomato puree)
35 ml/min (meat, milk, tomato, tomato
juice, ketchup)
45 ml/min (potato, frozen potato fries)

Initial Confirmatory Test

Injector Temperature: 210°C
Detector Temperature: 160°C
Column Temperature: 160°C
Nitrogen Carrier
Gas Flow: 45 ml/min

Gas Chromatographic
 Column: 6' x 4 mm packed with 28% Pennwalt
 223 + 4% KOH on 80/100 Gas Chrom R

GC CONDITIONS (typical)

Injector Temperature: 225°C
 Detector Temperature: 205°C
 Column Temperature: 115°C
 Nitrogen Carrier
 Gas Flow: 45 ml/min
 Chart Speed: 1 cm/min

EN-CAS Analytical Laboratories

REAGENTS

Water: Boiled MilliQ water
 MilliQ water purification system,
 Whatman, Whatman International, Ltd.,
 Maidstone, England

EDTA (tetrasodium): 98% purity, Mallinckrodt, Paris,
 Kentucky 40361

Hydrochloric Acid: Fisher Scientific, Fairlawn, NJ 07410

Stannous Chloride: Certified A.C.S., Fisher Scientific

Carbon Disulfide: Reagent grade, Fisher Scientific

Methanol: Pesticide, Fisher Scientific.

APPARATUS

Waring Blender: Not used

Reitz disintegrator: Hobart meat grinder used

Detector Temperature: 95°C
Column Temperature: 235°C
Nitrogen Carrier
Gas Flow: 15 ml/min

Tracor 540

Injector Temperature: 200°C
Detector Temperature: 80°C
Column Temperature: 215°C
Nitrogen Carrier
Gas Flow: 0.4 ml/min

Microtek 220 (initial confirmatory test)

Injector Temperature: 260°C
Detector Temperature: 255°C
Column Temperature: 140°C
Nitrogen Carrier
Gas Flow: 35 ml/min
Recorder Speed: 0.25 in/min

Morse Laboratories

REAGENTS

Water: Boiled deionized water
EDTA (tetrasodium): Mallinckrodt, Paris, Kentucky, 40361

EDTA: over 99%, Mallinckrodt
Paris, Kentucky 40361

EDTA Solution: 10% (v/v) in boiled deionized water

Hydrochloric Acid: Concentrated, EM Science, P.O. Box 70,
480 Democrat Rd., Gibbstown, NJ 08027

Stannous Chloride: Analytical grade, Mallinckrodt

Methanol: B & J, Baxter Healthcare Corp,
Muskegon, MI 49442

APPARATUS

Gas tight syringes: 10, 50, 100, 250, 500, 1000, 2500 ul,
Hamilton Co., Reno, NV

Monoject 3cc lock syringe,
Sherwood Medical, St. Louis, MO 63101

Gas Chromatograph: Microtek MT 220 Gas Chromatograph with
Flame Photometric Detector in the sulfur
mode.
Tracor, Inc., 6500 Tracor Lane, Austin,
TX 78721

Tracor 540 Gas Chromatograph with Flame
Photometric Detector in the sulfur mode.
Tracor, Inc.

Gas Chromatographic
Column: 240 cm x 6 mm o.d. x 2 mm i.d. packed
with 28% Alltech 223 + 4% KOH on 80/100
Gas Chrom R

Gas Chromatographic
Column for Initial
Confirmatory Test: 180 cm x 6 mm o.d. x 4 mm i.d. packed
with Chromosorb 108 80/100 mesh

GC CONDITIONS (typical)

Microtek 220

Injector Temperature: 215°C

APPARATUS

Crimp seals with
silicone/teflon lined
septums:

Seals, no. 13214; Teflon/Silicon Discs,
no. 12720
Pierce, Rockford, Illinois 61105

Gas Chromatograph:

Microtek 220 Gas Chromatograph equipped
with a Meloy Flame Photometric detector
in the sulfur mode; Model MT-220 FPD-S.
Tracor, Inc., 6500 Tracor Lane,
Austin, TX 78721

Gas Chromatographic
Column:

6' x 1/4" o.d. x 5/32" i.d. packed with
1/28% Pennwalt 223 + 4% KOH on 80/100 Gas
Chrom R.

Gas Chromatographic
Column for Initial
Confirmatory Test:

6' x 1/4" o.d. x 5/32" i.d. packed with
Chromosorb 108 80/100 mesh.

GC CONDITIONS (typical)

Injector Temperature:	210°C	
Detector Temperature:	160°C	
Column Temperature:	115°C	(meat, milk, tomato)
	135°C	(potato)
Nitrogen Carrier Gas Flow:	20 ml/min	(tomato paste)
	35 ml/min	(meat, milk, tomato)
	45 ml/min	(potato)

APPARATUS

Crimp seals with
silicone/teflon lined
septums:

Seals, no. 13214; Teflon/Silicon Discs,
no. 12720
Pierce, Rockford, Illinois 61105

Gas Chromatograph:

Microtek 220 Gas Chromatograph equipped
with a Meloy Flame Photometric detector
in the sulfur mode; Model MT-220 FPD-S.
Tracor, Inc., 6500 Tracor Lane,
Austin, TX 78721

Gas Chromatographic
Column:

6' x 1/4" o.d. x 5/32" i.d. packed with
/28% Pennwalt 223 + 4% KOH on 80/100 Gas
Chrom R.

Gas Chromatographic
Column for Initial
Confirmatory Test:

6' x 1/4" o.d. x 5/32" i.d. packed with
Chromosorb 108 80/100 mesh.

GC CONDITIONS (typical)

Injector Temperature: 210°C

Detector Temperature: 160°C

Column Temperature: 115°C (meat, milk, tomato)
135°C (potato)

Nitrogen Carrier
Gas Flow:

20 ml/min (tomato paste)

35 ml/min (meat, milk, tomato)

45 ml/min (potato)