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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Amended Frozen Storage Stability Study Protocols
and Analytical Methodology for Maneb and ETU on Raw
Agricultural Commodities and Processed Commodities
[RCB No. 2535] - No Accession Number

FROM: Martin F. Kovacs, Jr. Ph.D., Chemist
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RCB's Conclusions Re: Submitted Revised
Protocols/Analytical Methodology

1. The revised analytical methodologies for the
"Determination of Maneb and ETU in Crops," Morse
Laboratories, Inc., Sacramento, CA 95825 dated June 15, 1987
are acceptable to RCB.

2. The revised June 19, 1987 draft storage stability
study protocol "Determination of the Stability of Maneb and
ETU Residues in Fruit and RAC's," Morse Laboratories, Inc.,
Sacramento, CA is acceptable pending registrant clarification
as to the identity of the fourth crop to be tested on the
matrix list for storage stability studies, i.e., spinach or
potatoes.

3. The revised June 19, 1987 draft storage stability study protocol "Determination of the Stability of Maneb and ETU Residues in Processed Commodities," Morse Laboratories, Inc., Sacramento, CA is acceptable provided the registrant submits residue data on the following apple processed fractions: fresh juice, cooked/canned juice, wet pomace, dry pomace, cooked/canned applesauce, and strained baby food.

4. The (MTF) Maneb Task Force has not addressed the outstanding protocol deficiencies cited in the May 28, 1987 RCB memorandum for the following protocols:

a. Draft protocol "Pilot Study for the Determination of the Stability of Maneb and Ethylene Thiourea Residues in Stored Frozen Samples of Milk, Eggs, Beef Tissues, and Poultry Tissues," prepared by Hazleton Laboratories America, Inc., Madison, WI 53704 dated March 24, 1987.

b. Draft protocol "Determination of the Stability of Maneb Residues in Stored Frozen Samples of Milk, Eggs, Beef Tissues, and Poultry Tissues," prepared by Hazleton Laboratories America, Inc., Madison, WI 53704 dated March 24, 1987.

Recommendations

1. In an addendum to the storage stability study protocol for RACs, the commodity potatoes should be added to the current matrix list of crops for which storage stability studies are to be conducted. However, if the registrant decides to include spinach on the mane label then spinach must be substituted for potatoes.

2. The registrant must submit a plan for residue data on the following apple processed fractions in a revised storage stability study protocol for processed commodities: fresh juice, cooked/canned juice, wet pomace, dry pomace, cooked/canned applesauce, and strained baby food.

3. The registrant must submit revised protocols for animal commodity storage stability studies to address the outstanding deficiencies cited in RCB's May 28, 1987 memorandum.

4. This review should be sent to the petitioner as soon as possible so that our comments and suggestions may be incorporated into final protocols for frozen storage stability studies on raw agricultural, processed food, and animal commodities.

Background

The Maneb Task Force (MTF) has submitted the following revised storage stability study protocols and analytical methodology for both manebe and ETU in response to both RCB's M.F. Kovacs, Jr., May 28, 1987 memorandum re: "Evaluation of Frozen Storage Stability Study Protocols for Maneb and ETU on RAC's, Processed Commodities and Animal Products" and a subsequent meeting between RCB staff and representatives of the MTF on May 29, 1987 to discuss RCB's recommended revisions to the protocols evaluated in the aforementioned memorandum:

1. Draft storage stability study protocol "Determination of the Stability of Maneb and Ethylene Thiourea Residues in Fruit and Vegetable Raw Agricultural Commodities," prepared by Morse Laboratories, Inc., Sacramento, CA 95825 dated June 19, 1987.
2. Draft storage stability study protocol "Determination of the Stability of Maneb and Ethylene Thiourea Residues in Processed Commodities," prepared by Morse Laboratories, Inc., Sacramento, CA 95825 dated June 19, 1987.
3. Revised analytical method dated June 15, 1987 for "Determination of Maneb in Crops," Morse Laboratories, Inc., Sacramento, CA 95825.
4. Revised analytical method dated June 15, 1987 for "Determination of Ethylene Thiourea in Crops," Morse Laboratories, Inc., Sacramento, CA 95825.

However, the MTF has not submitted revised storage stability study protocols for the following as recommended by RCB in the June 28, 1987 memorandum:

1. Draft protocol "Pilot Study for the Determination of the Stability of Maneb and Ethylene Thiourea Residues in Stored Frozen Samples of Milk, Eggs, Beef Tissues, and Poultry Tissues," prepared by Hazleton Laboratories America, Inc., Madison, WI 53704 dated March 24, 1987.
2. Draft protocol "Determination of the Stability of Maneb Residues in Stored Frozen Samples of Milk, Eggs, Beef Tissues, and Poultry Tissues," prepared by Hazleton Laboratories America, Inc., Madison, WI 53704 dated March 24, 1987.

RCB has been asked by the Data Call-In (DCI) staff to review the aforesaid submitted revised analytical methodology/protocols to determine their acceptability as per EPA's March 31, 1987 DCI Notice for Maneb Storage Stability/Residue Data.

The recommended protocol revisions as delineated in RCB's May 28, 1987 memorandum are given below and are followed by the registrants original remarks, RCB's original comments in the May 28, 1987 memorandum, the registrant's current remarks, and RCB's current comments.

Present Considerations

I. DRAFT STORAGE STABILITY STUDY PROTOCOL FOR MANEB AND ETU ON RAW AGRICULTURAL COMMODITIES (RACS)

A. Test Matrix

Registrant's Original Remarks

This study will be conducted on apples, spinach, lettuce, and tomatoes.

RCB's Original Comments

We have no objections to test matrix

Registrant's Current Remarks

The study will be conducted on apples, lettuce, and tomatoes.

RCB's Current Comments

RCB's comments/conclusions paragraph of the M.F. Kovacs, Jr., June 26, 1986 memorandum re: "Protocol Change to Maneb and ETU Storage Stability Studies" in the March 31, 1987 Maneb DCI stated "RCB has no objections to the Maneb Task Force's selection of potatoes as an alternative crop, to spinach, for conducting the RAC storage stability studies as required by the 3/31/87 Maneb DCI."

This memorandum was prompted by the June 16, 1987 letter by Joseph D. Panetta, Chairman, MTF to Joan Warshawsky EPA/RD/SRB requesting replacement of spinach in the RAC storage stability study protocol with potatoes since the MTF had decided not to include spinach on the mane label and therefore not conduct any residue work pertaining to spinach.

RCB recommends that the commodity "potatoes" be added to the current Matrix list of crops to be studied. However, if the registrant (MTF) subsequently decides to include spinach on the maneb label then spinach must be substituted for potatoes in the Matrix of commodities to be studied.

1. Preparation and Storage of Fortified Control Samples

Registrant's Original Remarks

These "clean" controls will be used in the study. Sufficient "clean" control will be processed (ground) to provide enough thoroughly mixed sample for analysis of the analytes at various designated intervals during the study and two back-ups. Freezer storage will be conducted at 0 to 10 °F (-12 to -18 °C).

RCB's Original Comments

However, control samples should not be processed or ground and then set aside for weeks, months, etc., before fortification. The overall enzymatic strength may differ with time. Whole bulk control samples should be stored at -20 °C until ready for fortification.

Registrant's Current Remarks

These "clean" controls will be used in the study. Sufficient "clean" unground control will be divided into 17 subsamples. Each subsample will be individually stored frozen. All 17 subsamples will contain at least 5 units of RACs (fruit, heads, and bunches). Sixteen subsamples will be used for method controls and method spikes at the designated analysis intervals for both lab fortified and weathered residue stability studies (with four serving as back-ups) and will remain stored frozen and unground until needed. One frozen subsample (at least 4 lb) will be processed (ground or chopped) to allow for preparation of the stability spikes and two back-ups. (Freezer storage will be conducted at -20 ± 2 °C.)

RCB's Current Comments

RCB's original concerns regarding control sample grinding and temperature of storage have been addressed.

2. Maneb Stability in Fortified Samples

Registrant's Original Remarks

Maneb and ethylene thiourea will be analyzed at various intervals on samples fortified with maneb only. To accomplish this, two sets of samples will be fortified with maneb--one set for maneb analysis, the other set for ETU analysis. All fortifications will done at 2.0 ppm.

a. Preparation of maneb fortified samples for maneb determination: One g of frozen control sample will be placed in a 160 mL reaction bottle. Fifty mL of a maneb suspension in water at a concentration of 40 ug/mL

b. Preparation of maneb fortified sample for ethylene thiourea determination: Fifty g of frozen control sample will be placed in a 1 pt Mason jar. Two and one-half mL of a maneb suspension in water at a concentration of 40 ug/mL

RCB's Original Comments

In the May 28, 1987 memorandum: "One possible approach for fortification in the storage stability work is to chop the bulk samples. Then, immediately after chopping, samples could be weighted into suitable sample sizes for subsequent fortification with maneb or ETU at appropriate levels and then stored in the freezer at -20 °C until analyzed.

At the May 29, 1987 meeting with MTF: The protocol indicates that a 1-g size sample will be used for the maneb analysis. The Agency recommends that a larger sample be used to reduce potential quantitative analytical errors. G. Westburg noted that while larger samples (50 g) may be used in the CS₂ method, the method used in mancozeb analyses, the analytical method for maneb, using the GC head space procedure was developed using a 1-g sample. The samples intended for maneb analysis are double ground to assure representative sampling. While double grinding was of concern to the Agency, it was noted that this degree of maceration was not used in the ETU analysis which uses a larger sample, 50 g. When asked if the Agency would accept a 1-g sample for the maneb analyses, RCB representatives stated that the precision and accuracy of the method must be demonstrated to accommodate this small sample size.

Registrant's Current Remarks

Maneb and ethylene thiourea will be analyzed at various intervals on samples fortified with maneb only. To

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accomplish this, two sets of samples will be fortified with maneb--one set for maneb analysis, the other set for ETU analysis. All fortifications will be done at 2.0 ppm. All samples prepared for maneb analysis will undergo grinding (fine grind) while frozen with dry ice just prior to spiking. All samples prepared for ETU analysis will undergo grinding (coarse grind or chop) while frozen with dry ice just prior to spiking. The samples will remain frozen at all times until after addition of extraction solvents.

a. Preparation of maneb fortified samples for maneb determination: Four g of frozen, ground control sample will be placed in a 160 mL reaction bottle. Two hundred uL of a maneb suspension in water at a concentration of 40 ug/mL will be dispersed over the sample and allowed to soak in. The bottle will then be crimp sealed with a teflon-lined septum and immediately placed in the freezer.

b. Preparation of maneb fortified sample for ethylene thiourea determination: Fifty g of frozen, ground (chopped) control sample will be placed in a 1 pt Mason jar. Two and one-half mL of a maneb suspension in water at a concentration of 40 ug/mL will be dispersed over the sample and allowed to soak in. The jar will then be sealed with an aluminum foil lined lid and placed immediately in the freezer. Note: Above underlined text indicates revisions.

RCB's Current Comments

RCB concurs with the registrant's use of dry ice prior to sample spiking, the increase in sample size from 1 to 4 g for maneb analysis, and the proposed grinding process.

3. Ethylene Thiourea Stability in Fortified Samples

Registrant's Original Remarks

Ethylene thiourea will be analyzed at various intervals on samples fortified with ethylene thiourea only. All fortifications will be done at 2.0 ppm.

Preparation of ethylene thiourea fortified samples for ethylene thiourea determination: Fifty g of frozen control sample will be placed in a 1 pint Mason jar. Two and one-half mL of a ethylene thiourea solution in water at a concentration of 40 ug/mL will be dispersed over the sample and allowed to soak in. The jar will then be sealed with an aluminum foil-lined lid and placed immediately in the freezer.

RCB's Original Comments

In the May 28, 1987 memorandum: "RCB recommends that a fortification level of 0.5 ppm would be more appropriate. This level would be more consistent with expected residue levels in RACs. RCB also recommends that just prior to fortification replicate samples of chunks or pieces of each crop in a frozen state be prepared from whole bulk samples, and fortified frozen with ETU."

At the May 29, 1987 meeting with MTF: When spiking is part of the approved storage stability protocol, the Agency recommends that the fortification with ETU be conducted at a lower level than that proposed in the submitted protocol, i.e., 0.5 ppm should be used rather than the 2 ppm proposed.

Registrant's Current Remarks

Ethylene thiourea stability in fortified samples--Ethylene thiourea will be analyzed at various intervals on samples fortified with ethylene thiourea only. All fortifications will be done at 0.5 ppm.

Preparation of ethylene thiourea fortified samples for ethylene thiourea determination: Fifty g of frozen, ground (chopped) control sample will be placed in a 1 pt Mason jar. One mL of a ethylene thiourea solution in water at a concentration of 25 mg/mL will be dispersed over the sample and allowed to soak in. The jar will then be sealed with an aluminum foil-lined lid and placed immediately in the freezer. Note: Above underlined text indicates revisions.

RCB's Current Comments

RCB concurs with the registrants lowering the spiking level for ETU from 2.0 to 0.5 ppm and other underlined revisions shown above.

4. Sampling Intervals/Sampling for Fortified Samples

Registrant's Original Remarks

After the samples are fortified and stored, they will be analyzed at intervals of 0 day, 2 weeks, 1 month, 3 months, 6 months, and 12 months. Fortified samples for two additional intervals will also be prepared and stored to

serve as back-ups. The 0-day sample will be analyzed without being frozen. Two fortified samples (stability spikes), one method control, and one method spike (2.0 ppm) will be analyzed for each compound for each storage interval for each matrix.

RCB's Original Comments

RCB recommends that a minimum of five samples should be analyzed at each sampling interval; two stability spikes, one method control, and two method spikes.

Registrant's Current Remarks

Analysis scheme--After the samples are fortified and stored, they will be analyzed at intervals of 0 day, 2 weeks, 1 month, 3 months, 6 months, and 12 months. Fortified samples for two additional intervals will also be prepared and stored to serve as back-ups. Two fortified samples (stability spikes), one method control, and two method spikes (2.0 ppm for maneb and 0.5 ppm for ETU) will be analyzed for each compound for each storage interval for each matrix.

Note: Above underlined text indicates revisions.

RCB's Current Comments

RCB concurs with the registrant's revised analysis protocol to include five rather than four analyses at each sampling interval.

5. Analytical Methods

Registrant's Original Remarks

Analytical Methods--Maneb residues will be determined by the procedure entitled "Determination of Maneb in Crops, Morse Labs., Inc., 2/19/86."

Ethylene thiourea residues will be determined by the procedure entitled "Determination of Ethylene Thiourea in Crops, Morse Labs., Inc., 11/12/86."

RCB's Original Comments

In the May 28, 1987 memorandum: "No objections to overall principle of both methods. However, in the maneb method RCB recommends that samples not be doubly ground as indicated under Sample preparation. Excessive grinding operations could produce breakdown of the compounds being

analyzed due to generated heat, etc. In addition, larger than 1 gram sample portions should be utilized for sample analysis as indicated under Extraction paragraph. To accomplish larger sample sizes, scaled-up equipment may be needed as for eggs, milk, etc. This would eliminate the use of a Waring blender step in the sample preparation procedure. These analytical procedures must be properly validated via recovery runs with each test substrate at each sampling interval."

At the May 29, 1987 meeting with MTF: The protocol indicates that a 1-g size sample will be used for the maneb analysis. The Agency recommends that a larger sample be used to reduce potential quantitative analytical errors. G. Westburg noted that while larger samples (50 g) may be used in the CS₂ method, the method used in mancozeb analyses, the analytical method for maneb, using the GC head space procedure was developed using a 1-g sample. The samples intended for maneb analysis are double ground to assure representative sampling. While double grinding was of concern to the Agency, it was noted that this degree of maceration was not used in the ETU analysis which uses a larger sample, 50 g. When asked if the Agency would accept a 1-g sample for the maneb analyses, RCB representatives stated that the precision and accuracy of the method must be demonstrated to accommodate this small sample size.

Registrant's Current Remarks

Analytical Methods--Maneb residues will be determined by the procedure entitled "Determination of Maneb in Crops, Morse Labs., Inc., 6/15/87."

Ethylene thiourea residues will be determined by the procedure entitled "Determination of Ethylene Thiourea in Crops, Morse Labs., Inc., 6/15/87."

A revised analytical method dated June 15, 1987 for "Determination of Maneb in Crops," Morse Laboratories, Inc., Sacramento, CA 95825 was submitted. Only the revised portions of the original protocol dated February 19, 1986 are indicated below:

DATE: 12/4/85

Revision Date: 6/15/87

Reason for Revision: Incorporation of
4 gram sample size.

Maneb: Place 0.1 g Maneb in a 250 ml Erlenmeyer flask. Add 100 ml deionized water with a volumetric pipette. Cap and swirl vigorously. Place flask on a magnetic stirrer and add a magnetic stir bar. While the suspension is being mixed remove 1.0 ml and dilute to 100 ml with deionized water, making a 10 ug/ml solution. Remove 1.35 ml from the 10 ug/ml suspension while it is being rapidly stirred and add it to a 160 ml reaction flask. Add 8.65 ml 10% EDTA solution and 15 ml HCl/Stannous Chloride reagent to the reaction flask, and immediately seal it. React the flask in the same fashion as samples, as discussed later. Resulting concentration of prepared Maneb standard for gas chromatography is 0.1 ug/ml headspace.

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 of ground sample (see discussion). Add 15.0 ml HCl/Stannous Chloride (8N/3%) reagent. Immediately seal. Place flask in boiling water bath for 1 hour, hand

shaking flasks approximately every 5 minutes. After reaction, maintain sample at 100°C in the water bath during GLC analysis. Some crops, such as corn forage, may require reaction for two hours at the reaction temperature. This modification is only needed on matrixes where the procedure produces apparent poor recoveries at the standard reaction time of one hour.

Sample Fortification:

Make a 10 ug/ml standard as above under standard preparation. Place this suspension on a magnetic stirrer and add a stir bar. While the suspension is being mixed 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 mixed with a magnetic stirrer. Use only pipettes 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 fortifications at 100°C for the same time as the samples.

DISCUSSION:

The volume of 4.0 g of matrix is determined by weighing 4.0 g of sample into a 10 ml graduated cylinder and adding 5.0 ml water. The total volume minus 5.0 ml is the volume of the 4 g sample.

A revised analytical method dated June 15, 1987 for "Determination of Ethylene Thiourea in Crops," Morse Laboratories, Inc., Sacramento, CA 95825 was submitted. The following was the only change from the original method dated November 12, 1986:

Revision Date: 6/15/87

Reason for Revision: To delete washing of Gas Chrom S and change volume of ethanol addition from 1 to 4 ml at evaporation stage in cleanup.

RCB's Current Comments

In the revised analytical method for maneb dated June 15, 1987, the registrant has adequately addressed RCB's original concerns of: 1) small (1 g) sample sizes taken for analysis and 2) elimination of excessive sample grinding which could generate excessive heat leading to accelerated breakdown of compounds being analyzed by: 1) increasing sample size for maneb analyses from 1 to 4 g and 2) requiring sample grinding in the presence of dry ice and requiring further grinding of samples in dry ice at the discretion of the analyst and with proper documentation.

B. Storage Stability Using Weathered Samples

Registrant's Original Remarks

Sample Preparation--The entire sample received (approximately 2 lb) will be ground frozen (same method used on residue studies) and thoroughly mixed. Aliquots of ground sample will be removed for "0" time analysis. The remaining bulk, ground sample will be returned to the sample bag in which it was delivered and returned to the freezer.

Storage containers and storage conditions--Storage containers will be the sample bags (polyethylene lined residue bags) in which the samples were delivered. Freezer storage will be conducted at 0 to 10 °F (-12 to -18 °C).

RCB's Original Comments

The new procedure should include the storage of frozen samples at -20 °C in a whole unground condition prior to preparation for analyses.

Registrant's Current Remarks

Sample Preparation--The entire sample received (approximately 20 to 25 lb) will be divided into eight subsamples, each subsample containing at least five units (fruit, heads, or bunches) of RAC. Each subsample will be individually stored frozen and remain unground until analysis.

Storage containers and storage conditions--Storage containers will be the sample bags (polyethylene-lined residue bags) in which the samples were delivered. Freezer storage will be conducted at -20 ± 2 °C.

RCB's Current Comments

The registrant has complied with RCB's original request to store whole unground samples at -20 °C until analysis.

Registrant's Original Remarks

Analysis Scheme--At each analysis interval, the bulk sample will be removed from the freezer, allowed to partially thaw to allow for thorough mixing, have sample aliquots removed, then be returned to the freezer. The sample will be analyzed in duplicate along with a method control and method spike (2.0 ppm) for each storage interval for each matrix.

RCB's Original Comments

. . . RCB objects to the procedure of thawing and refreezing bulk field samples at each sampling interval. A better procedure would involve preparation of individual samples which are removed at each sampling interval to avoid thawing and refreezing of bulk-residue samples. RCB has no objection to method spike level. RCB recommends that a minimum of five samples be analyzed at each sampling interval: two stability spikes, one method control, and two method spikes.

Registrant's Current Comments

Analysis Scheme--Analysis will be conducted at intervals of 0 day, 2 weeks, 1 month, 3 months, 6 months, and 12 months. At each analysis interval, one of the subsamples will be removed from the freezer, finely ground frozen with dry ice and analyzed. The sample will be analyzed in duplicate along with a method control and two method spikes (2.0 ppm) for each storage interval for each matrix.

RCB's Current Comments

The registrant has complied with RCB's original request to avoid the refreezing of bulk field samples and analyzing five samples at each analysis interval.

II. DRAFT STORAGE STABILITY STUDY PROTOCOL FOR MANEB AND ETU IN PROCESSED COMMODITIES

A. Test Matrix

Registrant's Original Remarks

It is proposed that this study will be conducted on the following samples under the heading "Proposed representative samples for analysis":

<u>Crop</u>	<u>Processed Commodity Generated</u>	<u>Proposed Representative Samples for Analysis</u>
Apple	Fresh juice Cooked/canned juice Wet pomace Dry pomace Cooked/canned applesauce Strained baby food	Fresh juice Cooked & canned applesauce Wet pomace
Green Beans	Cannery waste Cooked & frozen beans Cooked & canned beans Cooked & pureed baby food	Cannery waste Cooked & canned beans
Potatoes	Cooked & dehydrated flakes/granules Cooked fried chips	Cooked fried chips
Tomatoes	Wet pomace Dry pomace Fresh juice Cooked canned juice Canned stewed tomatoes Canned pureed tomatoes Canned tomato sauce Canned tomato paste Canned tomato catsup	Wet pomace Fresh juice Canned stewed tomatoes Canned tomato sauce

Grapes	Wet pomace Dry pomace Fresh juice Raisins Raisin waste	Wet pomace Fresh juice Raisins
Sugar Beets	Crystalline beet sugar Dehydrated sugar beet pulp Sugar beet molasses	Crystalline beet sugar Dehydrated sugar beet pulp

RCB's Original Comments

In the May 28, 1987 memorandum: "RCB rejects the proposal by the petitioner that the protocol reflect data generated from representative processed commodities from a given crop study as being sufficient to show stability on the remaining processed commodities from that crop. The March 31, 1987 DCI Notice for maneb clearly states:

Repeated processing studies must be accompanied by frozen storage stability data for those repeated studies for both maneb and ETU on each processed commodity derived from each submitted processing study. Furthermore, frozen storage stability data must be submitted to support the sugar beet processing studies.

"RCB recommends that the following processed commodities be added to the list for analysis: dry apple pomace, cooked/canned applesauce, strained applesauce (baby food), cooked and frozen beans, cooked and pureed beans (baby food), cooked and dehydrated potato flakes/granules, dry tomato pomace, cooked canned tomato juice, canned pureed tomatoes, canned tomato paste and catsup, dry grape pomace, raisin waste, and sugar beet molasses."

At the May 29, 1987 meeting with MTF: The protocol proposed that only certain representative commodity samples be analyzed to comply with the residue chemistry requirements. Dr. Kovacs stated that because of the nature of EBDC/ETU it was recommended that all of the processed commodities identified in the review be analyzed although this is more stringent than the usual Agency requirements.

Registrant's Current Remarks

It is proposed that this study will be conducted on the following samples under the heading "Processed Commodity Generated":

<u>Crop</u>	<u>Processed Commodity Generated</u>
Green Beans	Cannery waste Cooked & frozen beans Cooked & canned beans Cooked & pureed baby food
Potatoes	Cooked & dehydrated flakes/granules Cooked fried chips
Tomatoes	Wet pomace Dry pomace Fresh juice Cooked canned juice Canned stewed tomatoes Canned pureed tomatoes Canned tomato sauce Canned tomato paste Canned tomato catsup
Grapes	Wet pomace Dry pomace Fresh juice Raisins Raisin waste
Corn	Cannery waste
Sugar beet	Crystalline beet sugar Dehydrated sugar beet pulp Sugar beet molasses

RCB's Current Comments

The registrant has complied with RCB's original request for all processed commodities generated from green beans, potatoes, tomatoes, grapes, and sugar beets including

the registrant's own addition of corn cannery waste. However, all processed apple commodities originally proposed for analysis by the registrant [fresh juice, cooked and canned applesauce and wet pomace) as well as the additional commodities dry apple pomace, and strained applesauce (baby food) recommended by RCB in its May 28, 1987 memorandum; have been deleted from the final protocol. The deletion of all processed apple commodities for residue analysis is not acceptable to RCB.

B. Control Sample Suitability Determination

Registrant's Original Remarks

Control (untreated or store-bought) samples of each matrix will be analyzed prior to initiation of study to assure they are not contaminated with either maneb or ethylene thiourea above the detection limit of the analytical procedures. Should residues be detected, screening of controls will continue until uncontaminated samples are found. These "clean" controls will be used in the study. Sufficient "clean" control will be processed (ground) to provide enough thoroughly mixed sample for analysis of the analytes at various designated intervals during the study and two back-ups.

Storage containers will be the actual containers in which the analytical extraction occurs. This eliminates possible losses due to adsorption onto glass or excess manipulation of the small amount of stored samples. Freezer storage will be conducted at 0 to 10 °F (-12 to -18 °C).

RCB's Original Comments

However, control samples should not be processed or ground and then set aside for weeks, months, etc., before fortification. The overall enzymatic strength may differ with time. Whole bulk control samples should be stored at -20 °C until ready for fortification.

Registrant's Current Remarks

Sufficient "clean" unground control will be divided into nine subsamples. Each subsample will be individually stored frozen. Eight subsamples, each containing at least 1/2 lb processed commodity, will be used for method controls and method spikes at the designated analysis intervals (with two serving as back-ups) and will remain stored frozen and unground until needed. One frozen subsample containing at least 4 lb processed commodity will be ground or chopped, where applicable, to allow for the preparation of the

stability spikes and two back-ups. (Freezer storage will be conducted at -20 ± 2 °C.)

RCB's Current Comments

RCB's original concerns regarding control sample grinding and temperature of storage have been addressed.

C. Maneb Stability in Fortified Samples

Registrant's Original Remarks

Maneb and ethylene thiourea will be analyzed at various intervals on samples fortified with maneb only. To accomplish this, two sets of samples will be fortified with maneb--one set for maneb analysis, the other set for ETU analysis. All fortifications will done at 2.0 ppm.

Preparation of maneb fortified samples for maneb determination: One g of frozen control sample will be placed in a 160 mL reaction bottle. Fifty uL of a maneb suspension in water at a concentration of 40 ug/mL

Preparation of maneb fortified sample for ethylene thiourea determination: Fifty g of frozen control sample will be placed in a 1 pt Mason jar. Two and one-half mL of a maneb suspension in water at a concentration of 40 ug/mL

RCB's Original Comments

In the May 28, 1987 memorandum: "One possible approach for fortification in the storage stability work is to chop the bulk samples. Then, immediately after chopping, samples could be weighed into suitable sample sizes for subsequent fortification with maneb or ETU at appropriate levels and then stored in the freezer at -20 °C until analyzed."

At the May 29, 1987 Meeting with MTF: The protocol indicates that a 1-g size sample will be used for the maneb analysis. The Agency recommends that a larger sample be used to reduce potential quantitative analytical errors. G. Westburg noted that while larger samples (50 g) may be used in the CS₂ method, the method used in mancozeb analyses, the analytical method for maneb, using the GC head space procedure was developed using a 1-g sample. The samples intended for maneb analysis are double ground to assure representative sampling. While double grinding was of concern to the Agency, it was noted that this degree of maceration was

not used in the ETU analysis which uses a larger sample, 50 g. When asked if the Agency would accept a 1-g sample for the maneb analyses, RCB representatives stated that the precision and accuracy of the method must be demonstrated to accommodate this small sample size.

Registrant's Current Remarks

Maneb and ethylene thiourea will be analyzed at various intervals on samples fortified with maneb only. To accomplish this, two sets of samples will be fortified with maneb--one set for maneb analysis, the other set for ETU analysis. All fortifications will be done at 2.0 ppm. All samples prepared for maneb analysis will undergo grinding (fine grind) while frozen with dry ice just prior to spiking. All samples prepared for ETU analysis will undergo grinding (coarse grind or chop) while frozen with dry ice just prior to spiking. The samples will remain frozen at all times until after addition of extraction solvents.

a. Preparation of maneb fortified samples for maneb determination: Four g of frozen, ground control sample will be placed in a 160 mL reaction bottle. Two hundred uL of a maneb suspension in water at a concentration of 40 ug/mL will be dispersed over the sample and allowed to soak in. The bottle will then be crimp sealed with a teflon-lined septum and immediately placed in the freezer.

b. Preparation of maneb fortified sample for ethylene thiourea determination: Fifty g of frozen, ground (chopped) control sample will be placed in a 1 pt Mason jar. Two and one-half mL of a maneb suspension in water at a concentration of 40 ug/mL will be dispersed over the sample and allowed to soak in. The jar will then be sealed with an aluminum foil-lined lid and placed immediately in the freezer. Note: Above underlined text indicates revisions.

RCB's Current Comments

RCB concurs with the registrant's use of dry ice prior to sample spiking, the increase in sample size from 1 to 4 g for maneb analysis, and the proposed grinding process.

D. Ethylene Thiourea Stability in Fortified Samples

Registrant's Original Remarks

Ethylene thiourea will be analyzed at various intervals on samples fortified with ethylene thiourea only. All fortifications will be done at 2.0 ppm.

Preparation of ethylene thiourea fortified samples for ethylene thiourea determination: Fifty g of frozen control sample will be placed in a 1 pint Mason jar. Two and one-half mL of a ethylene thiourea solution in water at a concentration of 40 ug/mL will be dispersed over the sample and allowed to soak in. The jar will then be sealed with an aluminum foil-lined lid and placed immediately in the freezer.

RCB's Original Comments

In the May 28, 1987 memorandum: "RCB recommends that a fortification level of 0.5 ppm would be more appropriate. This level would be more consistent with expected residue levels in processed commodities. RCB also recommends that just prior to fortification replicate samples of chunks or pieces of each processed commodity in a frozen state be prepared from whole bulk samples, and fortified frozen with ETU."

At the May 29, 1987 meeting with MTF: When spiking is part of the approved storage stability protocol, the Agency recommends that the fortification with ETU be conducted at a lower level than that proposed in the submitted protocol, i.e., 0.5 ppm should be used rather than the 2 ppm proposed.

Registrant's Current Remarks

Ethylene thiourea stability in fortified samples--Ethylene thiourea will be analyzed at various intervals on samples fortified with ethylene thiourea only. All fortifications will be done at 0.5 ppm.

Preparation of ethylene thiourea fortified samples for ethylene thiourea determination: Fifty g of frozen, ground (chopped) control sample will be placed in a 1 pt Mason jar. One mL of a ethylene thiourea solution in water at a concentration of 25 ug/mL will be dispersed over the sample and allowed to soak in. The jar will then be sealed with an aluminum foil lined lid and placed immediately in the freezer. Note: Above underlined text indicates revisions.

RCB's Current Comments

RCB concurs with the registrant's lowering the spiking level for ETU from 2.0 to 0.5 ppm and other changes.

E. Analysis Scheme

Petitioner's Original Remarks

After the samples are fortified and stored, they will be analyzed at intervals of 0 day, 2 weeks, 1 month, 3 months, 6 months, and 12 months. Fortified samples for two additional intervals will also be prepared and stored to serve as back-ups. The 0-day sample will be analyzed without being frozen. Two fortified samples (stability spikes), one method control and one method spike (2.0 ppm) will be analyzed for each compound for each storage interval for each matrix.

RCB's Original Comments

RCB recommends that a minimum of five samples should be analyzed at each sampling interval: two stability spikes, one method control, and two method spikes.

Registrant's Current Remarks

Analysis scheme--After the samples are fortified and stored, they will be analyzed at intervals of 0 day, 2 weeks, 1 month, 3 months, 6 months, and 12 months. Fortified samples for two additional intervals will also be prepared and stored to serve as back-ups. Two fortified samples (stability spikes), one method control and two method spikes (2.0 ppm for maneb and 0.5 ppm for ETU) will be analyzed for each compound for each storage interval for each matrix. Note: Above underlined text indicates revisions.

RCB's Current Comments

RCB concurs with the registrant's revised analysis protocol to include five rather than four analyses at each sampling interval.

Analytical Methods

Registrant's Original Remarks

Analytical Methods--Maneb residues will be determined by the procedure entitled "Determination of Maneb in Crops, Morse Labs., Inc., 2/19/86."

Ethylene thiourea residues will be determined by the procedure entitled "Determination of Ethylene Thiourea in Crops, Morse Labs., Inc., 11/12/86."

RCB's Original Comments

In the May 28, 1987 memorandum: "No objections to overall principle of both methods. However, in the maneb method RCB recommends that samples not be doubly ground as indicated under Sample preparation. Excessive grinding operations could produce breakdown of the compounds being analyzed due to generated heat, etc. In addition, larger than 1 gram sample portions should be utilized for sample analysis as indicated under Extraction paragraph. To accomplish larger sample sizes, scaled-up equipment may be needed as for eggs, milk, etc. This would eliminate the use of a Waring blender step in the sample preparation procedure. These analytical procedures must be properly validated via recovery runs with each test substrate at each sampling interval."

At the May 29, 1987 meeting with MTF: The protocol indicates that a 1-g size sample will be used for the maneb analysis. The Agency recommends that a larger sample be used to reduce potential quantitative analytical errors. G. Westburg noted that while larger samples (50 g) may be used in the CS₂ method, the method used in mancozeb analyses, the analytical method for maneb, using the GC head space procedure was developed using a 1-g sample. The samples intended for maneb analysis are double ground to assure representative sampling. While double grinding was of concern to the Agency, it was noted that this degree of maceration was not used in the ETU analysis which uses a larger sample, 50 g. When asked if the Agency would accept a 1-g sample for the maneb analyses, RCB representatives stated that the precision and accuracy of the method must be demonstrated to accommodate this small sample size.

Registrant's Current Remarks

Analytical Methods--Maneb residues will be determined by the procedure entitled "Determination of Maneb in Crops, Morse Labs., Inc., 6/15/87."

Ethylene thiourea residues will be determined by the procedure entitled "Determination of Ethylene Thiourea in Crops, Morse Labs., Inc., 6/15/87."

The revisions to the subject analytical methods for maneb and ETU have been described earlier in this review under I.A.5. Analytical Methods.

RCB 's Current Comments

In the revised analytical method for maneb dated June 15, 1987, the registrant has adequately addressed RCB's original concerns of: 1) small (1 g) sample sizes taken for analysis and 2) elimination of excessive sample grinding which could generate excessive heat leading to accelerated breakdown of compounds being analyzed by: 1) increasing sample size for maneb analyses from 1 to 4 g and 2) requiring sample grinding in the presence of dry ice and requiring further grinding of samples in dry ice at the discretion of the analyst and with proper documentation.

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