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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Evaluation of Frozen Storage Stability Study Protocols
for Maneb and ETU on Raw Agricultural Commodities,
Processed Commodities, and Animal Products
[RCB No.2254] No Accession No.

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Introduction

In response to EPA's 3(c)(2)(B) Storage Stability/Residue Data Call-In (DCI) Notice for Maneb dated March 31, 1987, the Maneb Task Force (MTF) has submitted the following information/protocols for the Agency's evaluation:

1. Data transmittal memorandum from Gerald Frazee, Griffin Corporation to Ms. Joan Warshawsky, EPA, dated May 6, 1987.
2. 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 April 27, 1987.
3. 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 April 27, 1987.

4. 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.
5. 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.
6. Revised analytical method dated February 19, 1986 for "Determination of Maneb in Crops," Morse Laboratories, Inc. Sacramento, CA 95825.
7. Revised analytical method dated November 12, 1986 for "Determination of Ethylene Thiourea in Crops," Morse Laboratories, Inc. Sacramento, CA 95825.
8. APPENDIX A/B, "A Gas Chromatographic Method for Measurement of Dithane," by Hazleton Laboratories America, Inc., dated August 14, 1986.
9. APPENDIX A, "An Analytical Method for Determining Ethylene Thiourea in Chicken Tissues, Eggs, and Excreta," by Maneb Task Force (Rohm & Haas Company, Philadelphia, PA 19105) Method TR 36F-82-15, undated.

RCB has been asked by the DCI staff to review the aforementioned submitted information/protocols to determine their acceptability as per EPA's March 31, 1987 DCI Notice for Maneb Storage Stability/Residue Data.

Present Considerations

I. Data Transmittal Memorandum Dated May 6, 1987

A. Petitioner's Remarks (In Part)

1. "The Agency is aware that it is the intent of the Maneb Task Force to validate all of the results generated in the livestock/poultry feeding studies as well as the majority of the RAC field residue studies. We will be initiating new processed goods studies and running storage stability concurrently.
2. We are, however, concerned about ETU stability in spiked samples. Since it is impossible to accurately spike whole crops for storage stability analysis we propose to spike frozen ground samples with ETU. We believe these are the only conditions which will allow us to show ETU stability. We do, however, question the probative value of this

approach since the conditions of spiking are unrealistic relative to field application of maneb to the crop. Literature data strongly indicate ETU is unstable when exposed to plant matrices and/or ultraviolet light, thus making it extremely difficult, if not impossible, to show stability of a compound which is highly unstable under normal conditions.

3. We also wish to note that our chain of custody data are currently being compiled and tabulated so that actual storage conditions from prior studies can be repeated in the stability studies. This compilation will be made available to the Agency as soon as it is completed."

B. Our Comments

1. Since it is the Agency's decision that possible validation of existing crop residue, processing, and animal commodity samples would not be accepted, RCB cannot concur with the proposal by the MTF to validate the results of the livestock/poultry feeding studies as well as the majority of the RAC field residue studies. In fact, for the petitioner to pursue this course of action would be in direct conflict with the requirements of the Maneb DCI Notice for Storage Stability Data which states in part:

"The Agency has considered possible validation of earlier submitted data, but has concluded that validation of existing crop residue, processing, and animal commodity samples would not be acceptable due to the highly variable and in many instances unknown conditions (e.g., ambient and freezer temperatures, sample handling, preparation and extraction parameters prior to analyses) which may have existed at the residue laboratories generating the residue data which were evaluated. In order to accurately reflect storage conditions, the Agency has concluded that frozen storage stability data must be generated concurrently with the required residue, processing, and animal commodity studies."

The petitioner should be reappraised of the aforementioned DCI requirements. If the registrant attempts to validate any of the existing data, it would be at his own risk, but it is unlikely that the Agency will find these acceptable. Therefore, RCB's review of this protocol is directed toward storage stability studies designed to validate new data.

2. Many products such as apples, tomatoes, animal commodities, etc., may be stored in commercial chambers for several weeks/months at low temperature before use. Samples may also be stored in laboratory freezers for some time before residue analyses are carried out. In both instances, enzymatic effects on the maneb and ETU should be lessened by lowering the temperature. Therefore, for carrying out the present stability studies, it would be appropriate for the petitioner to put his control bulk samples into a -20 °C freezer. One possible approach for fortification in the storage stability work is to chop the bulk samples. Immediately after chopping (preparing) his bulk samples, he could then weigh out all of his prepared samples in suitable containers, fortify them at the appropriate levels, and store them in a freezer at -20 °C until ready for analyses.

The petitioner mentions that literature data strongly indicate ETU is unstable when exposed to plant matrices and/or ultraviolet light. This is true for many compounds, i.e., degradation/metabolic processes take place. However, one purpose of storage stability studies is to find some correlation as to how much degradation could take place when the treated field samples such as apples, tomatoes, etc., are placed in storage at various time intervals; this is independent with regard to the application of maneb to the crop in the field.

Since the petitioner intends to validate rather than repeat a majority of RAC field studies, his submitted draft storage stability study protocol for fruit and vegetable RACs discussed in detail below employs spiking of processed (ground) control

samples with both maneb and ETU. For validation purposes, this spiking protocol was presumably intended to duplicate or emulate the condition of many of the previously treated field residue samples (i.e., frozen and stored in a ground or macerated condition for several weeks/months).

RCB reiterates the Agency's decision that validation of previously submitted residue data which would entail fortification or spiking of frozen and ground residue samples with maneb and ETU is not only in conflict with the requirements of the March 31, 1987 DCI, but in addition based on published data in the scientific literature including storage stability data submitted to date by the MTF, would lead to unsupportable or inconclusive storage stability data for maneb/ETU in the sample matrices examined.

3. Since the Agency has decided not to accept storage stability studies designed to validate existing crop residue and animal feeding studies information provided by the petitioner on "chain of custody data" will not be required. The petitioner may submit this information at his own risk.

II. Draft Storage Stability Study Protocol for Maneb and ETU on RACs

Petitioner's Remarks

STUDY OBJECTIVE

"The objective of this study is to determine the stability of maneb and ethylene thiourea in raw agricultural commodities. This study is in support of raw agricultural commodity residue data previously generated by various laboratories for the Maneb Task Force in response to the October, 1984 and April, 1985 Data Call-In Notices. EPA is requiring the Maneb Task Force to analyze four crops in this study which will serve to represent all raw agricultural commodities analyzed for the data call-in. The four crops are apples, spinach, lettuce and tomatoes.

TEST MATRIX

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

TEST MATERIALS

Parent compound:	Maneb [[1,2 ethanediy]bis [carbamodithioate]](2-) manganese
Molecular formula:	C ₄ H ₆ MnN ₂ S ₄
Molecular weight:	265.29
Physical state:	Solid
Metabolite:	Ethylene thiourea (2-imidazolidinethione)
Molecular formula:	C ₃ H ₆ N ₂ S
Molecular weight:	102.17
Physical state:	Solid

Storage stability studies for maneb will be conducted on both weathered samples and laboratory fortified samples. Ethylene thiourea storage stability will be determined using laboratory fortified samples only."

Our Comments

We have no objections to test matrix, test materials, or overall conduct of the study, which are all consistent with the requirements of the March 31, 1987 DCI Notice for maneb. However, RCB does object to the statement that this study is in support of raw agricultural commodity data previously generated by various laboratories of the Maneb Task Force See RCB's comments above under Data Transmittal Memorandum Dated May 6, 1987.

A. Storage Stability Using Fortified Samples

1. Preparation and Storage of Control Samples

Petitioner's 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" controls 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)."

Our Comments

There is no objection to selection and pretesting of control samples. 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.

2. Maneb Fortification and Sample Preparation

Petitioner's 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.

a) Preparation of Maneb fortified samples for Maneb determination:

1.0 g of frozen control sample will be placed in a 160 ml reaction bottle. 50 microliters of a Maneb suspension in water at a concentration of 40 micrograms/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: 50.0 g of frozen control sample will be placed in a 1 pint Mason jar. 2.5 ml of a Maneb suspension in water at a concentration of 40 micrograms/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."

Our Comments

No objections to fortification levels, assuming: (1) this level is well above the limits of detection for both mane b and ETU by the analytical procedures which will be employed; and (2) the analytical methodologies used give adequate recoveries of these two compounds at this fortification level. 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 mane b or ETU at appropriate levels and then stored in the freezer at -20 °C until analyzed. However, RCB cannot recommend or instruct the petitioner to follow the preceding approach since he has the laboratory accessibility and must take the best approach for fortification wherein he is able to validate his new field residue data. The registrant is advised to analyze his field samples as quickly as possible to minimize decomposition during storage.

3. ETU Fortification and Sample Preparation

Petitioner's 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.

- a) Preparation of Ethylene Thiourea fortified samples for Ethylene Thiourea determination:
50.0 g of frozen control sample

will be placed in a 1 pint Mason jar. 2.5 ml of a Ethylene Thiourea solution in water at a concentration of 40 micrograms/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: Preliminary studies have shown that in the case of apples, Ethylene Thiourea has very little stability. When thawed samples are fortified with Ethylene Thiourea, only 10-20% is recovered after 1 day frozen storage. However when frozen samples are fortified with Ethylene Thiourea, 60% recovery has been attained after 8 days frozen storage. Although it appears fortifying frozen samples will provide better results, we feel this technique is not the proper way to conduct a stability study and will, therefore, continue to fortify thawed samples."

Our Comments

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.

The petitioner concludes that fortification of frozen and thawed samples will produce different results. RCB has not seen the detailed experimental results to substantiate this conclusion. RCB is recommending that the petitioner take his harvested bulk samples from the freezer, chop them, weigh and spike, and return the samples to the freezer. However, the petitioner is responsible for carrying out his storage stability studies using the best techniques that would ensure sample integrity and thus provide valid storage stability data necessary to validate new residue studies.

4. Sampling Intervals/Sampling

Petitioner's Remarks

"After the samples are fortified and stored, they will be analyzed at 0 day, 2 week, 1 month, 3 month, 6 month, and 12 month intervals. Fortified samples for two additional intervals will also be prepared and stored to serve as back-ups. The day 0 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."

Our 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.

5. Analytical Methods (See Items 6 and 7 above under Introduction)

Petitioner's Remarks

"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".

See methods attached to this protocol."

Our Comments

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.

B. Storage Stability Using Weathered Samples

1. Overall Conduct of Study

Petitioner's Remarks

"Maneb only will be analyzed for on samples containing weathered residue. The determinations will be conducted on all matrices at various intervals of frozen storage. Field treated crop samples will be frozen immediately upon harvesting. Samples will be kept frozen during transport to the laboratory. Upon receipt by the laboratory, analysis will be conducted as soon as is reasonably possible (within 1-2 days, allowing for sample log-in, sample processing and reagent preparation) to establish a 0 time residue level."

Our Comments

No objection. Overall conduct of study consistent with requirements of March 31, 1987 DCI Notice.

2. Preparation and Storage of Samples

Petitioner's Remarks

"The entire sample received (approximately 2 lbs.) 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 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)."

Our Comments

The sample preparation and storage procedure, as clearly indicated by the petitioner's comments, were designed to mimic or duplicate those conditions or procedures which existed during sample collection, preparation, and storage of the original residue samples (i.e., to validate the earlier residue studies). Since the Agency has decided above that protocol procedures designed to validate existing crop residue studies are not acceptable, new procedures must be developed. The new procedure should include the storage of frozen samples at -20 °C in a whole unground condition prior to preparation for analyses.

3. Sampling Intervals/Sampling

Petitioner's Remarks

"After the samples are ground and stored, they will be analyzed at 0 time, 2 week, 1 month, 3 month, 6 month, and 12 month intervals. The 0 time sample will be analyzed immediately after the sample is ground. 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 a method spike (2.0 ppm) for each storage interval for each matrix."

Our Comments

See RCB's comments under II.A.4. for fortification study. In addition, 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 (see RCB's comments under II.A.2. above.

4. Analytical Methods

Petitioner's Remarks

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

See method attached to this protocol."

Our Comments

See RCB's comments under II.A.5. above for maneb analytical procedure.

C. Study Reporting Requirements

Petitioner's Remarks

"The report will contain the following:

A description of the methods and procedures used for the determination of Maneb and ETU residues.

The results of samples analyzed, expressed in ppm and recovery where indicated, expressed in percent.

Recovery and control values for each analytical run.

A graphic display that shows results obtained versus time of storage for each matrix.

All aspects of this study carried out by Morse Laboratories, Inc. will be conducted in accordance with those EPA Pesticide Programs Good Laboratory Practice Standards (40 CFR 160) that, as interpreted by Morse Laboratories, Inc. apply to analytical laboratories.

All notebooks, worksheets, standard preparation data, logs and original chromatograms will be retained by Morse Laboratories, Inc. for a period of time agreed upon by Morse Laboratories, Inc. and the Maneb Task Force."

Our Comments

It appears that the petitioner is making adequate provision for recordkeeping in conjunction with the proposed study.

III. Draft Storage Stability Study Protocol for Maneb and ETU On Processed Commodities

A. Study Objective

Petitioner's Remarks

"The objective of this study is to determine the stability of Maneb and Ethylene Thiourea in processed commodities. This study is in support of processed residue data previously generated and to be generated by various laboratories for the Maneb Task Force in response to the October, 1984 and April, 1985 Data Call-In Notices. EPA is requiring the Maneb Task Force to determine stability on all processing studies. This protocol will be based on a proposal that data generated from representative processed commodities from a given crop study be sufficient to show stability on the remaining processed commodities from that crop."

Our Comments

The petitioner states that "this study is in support of processed residue data previously generated" (i.e., a validation study) whereas in the data transmittal memorandum dated May 6, 1987 it is clearly stated "We will be initiating new processed goods studies and running storage stability concurrently." These conflicting statements need to be resolved by the petitioner. RCB also 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."

B. Test Matrix

Petitioner's 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

<u>Crop</u>	<u>Processed Commodity Generated</u>	<u>Proposed representative samples for analysis</u>
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 Beet	Crystalline beet sugar Dehydrated sugar beet pulp Sugar beet molasses "	Crystalline beet sugar Dehydrated sugar beet pulp

Our Comments

For the reasons discussed above under III.3.A., 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.

C. Test Materials

Petitioner's Remarks

"Parent compound: Maneb [[1,2 ethanediylbis [carbamodithioate]](2-) manganese
Molecular formula: C₄H₆MnN₂S₄
Molecular weight: 265.29
Physical state: Solid
Metabolite: Ethylene Thiourea
(2-imidazolidinethione)

Molecular formula: C₃H₆N₂S
Molecular weight: 102.17
Physical state: Solid "

Our Comments

No objections.

D. Conduct of Study and Preparation and Storage of Controls

Petitioner's Remarks

"Storage stability studies for Maneb and Ethylene Thiourea will be conducted on laboratory fortified samples.

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 samples 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)."

Our Comments

No objection to overall conduct of study which is consistent with requirements of March 31, 1987 DCI Notice for Maneb. See RCB's comments under II.A.1. concerning preparation and storage of controls.

E. Maneb Fortification and Sample Preparation

Petitioner's Remarks

[Same as II.A.2. above for RAC protocol]

Our Comments

[Same as II.A.2. above for RAC protocol except no comment necessary for sample preparation prior to fortification.]

F. ETU Fortification and Sample Preparation

Petitioner's Remarks

[Same as II.A.3. above for RAC protocol]

Our Comments

[Same as II.A.3. above for RAC protocol except no comment necessary for sample preparation prior to fortification.]

G. Sampling Intervals/Sampling

Petitioner's Remarks

[Same as II.A.4. above for RAC protocol]

Our Comments

[Same as II.A.4. above for RAC protocol but modified to address processed commodities rather than RACs.]

H. Analytical Methods (See Items 6. and 7. above under Introduction)

Petitioner's Remarks

[Same as II.A.5. above for RAC protocol.]

Our Comments

No objection to overall principle of both methods; however, in the maneb method RCB recommends larger sample portions be utilized for sample extraction.

I. Study Reporting Requirements

Petitioner's Remarks

[Same reporting requirements as described above in II.C. for RAC protocol.]

Our Comments

[Same as II.C. above for RAC protocol.]

IV. Draft Pilot Study Protocol for Maneb and ETU Stability in Animal Commodities

A. Study Objective

Petitioner's Remarks

"The objective of this study is to determine the stability of Maneb and ethylene thiourea (ETU) in milk, eggs, and tissue samples stored frozen in screw-cap bottles from 0 to 10 days. This study is a "Pilot Study" in support of the following stability studies entitled "Determination of the Stability of Maneb Residues in Stored Frozen Samples of Milk, Eggs, Beef Tissues, and Poultry Tissues," and "Determination of Ethylene Thiourea Residues in Stored Frozen Samples of Milk, Eggs, Beef Tissues, and Chicken Tissues."

Our Comments

RCB disagrees with the stated objective of this "Pilot Study" (which in RCB's opinion is a preliminary and not an official final protocol) to support (i.e., validate) previously submitted dairy cattle and poultry feeding studies. The March 31, 1987 DCI Notice for mane b clearly states:

"In the absence of frozen storage stability data for stored animal commodities and in order that the Agency might conduct its residue assessment, the dairy cattle (meat and milk) and poultry (poultry and eggs) feeding studies must be repeated. The repeated animal feeding studies must be accompanied by frozen storage stability data for both Maneb and ETU on each commodity required to be examined."

B. Test Matrix

Petitioner's Remarks

This study will be conducted on eggs and poultry muscle and also on milk, liver, kidney, and fat of cattle.

Our Comments

No objections.

C. Test Materials

Petitioner's Remarks

" Compound:	Maneb [[1,2 ethanediybis [carbamodithioate]](2-7) manganese
Lot No.:	1A5026
Molecular formula:	$C_4H_6MnN_2S_4$
Molecular weight:	265.29
Physical state:	Solid
Compound:	Ethylene thiourea (2-imidazolidinethione)
Lot No.:	6711A
Molecular formula:	$C_3H_6N_2S$
Molecular weight:	102.17
Physical state:	Solid "

Our Comments

No objections.

D. Sample Amendment

Petitioner's Remarks

"Before the control samples are amended with Maneb and ETU, the spiking mixtures will be analyzed by HLA to verify the amount to be added to each sample.

Maneb. For each matrix, two samples will be amended with Maneb at the 500-ppb level. Kidney samples

will be amended at 1.0 ppm. One control of each matrix will accompany the amended samples. The samples will be blended and 10.0 g of sample will be weighed directly into screw-cap bottles for storage. The individual samples will be spiked frozen with 25 to 100 μ L of an appropriate level of suspended Maneb in H₂O.

ETU.

Two samples of each matrix will be fortified with ETU at 500 ppb. Three grams of a preblended sample will be weighed directly into screw-cap bottles for storage. The 3.0-g sample will be spiked frozen with 10 to 25 μ L of an appropriate level of diluted ETU in methanol."

Our Comments

No objections to fortification levels, assuming: (1) these levels are well above the limits of detection for both maneab and ETU by the analytical procedures which will be employed; and (2) the analytical methodologies used gives adequate recoveries of these two compounds at their respective fortification levels. RCB recommends that larger samples be used for ETU spiking rather than 3 g samples and that diced or cut-up tissue samples be fortified rather than homogenized samples.

E. Sampling Intervals/Sampling

Petitioner's Remarks

"After samples are amended, they will be analyzed at 0 and 10 days. The 0 day samples will be analyzed without being frozen. Two amended samples, one control, and one control spiked at an equivalent level, will be analyzed for each matrix and compared at 0 to 10 days.

The fortified Maneb and ETU samples to be analyzed after 10 days, including their respective controls, will be stored frozen at approximately -20°C in accordance with HLA SOP OP-GENC 21. Each sample will be assigned a unique HLA number for identification."

Our Comments

Since the intent of this "pilot study" is not clear to RCB, the relevancy of the short duration of 10 days cannot be commented upon. RCB recommends, however, a minimum of five samples (each of mane b and ETU) should be analyzed at each sampling interval; two stability spikes, one method control, and two method spikes.

F. Analytical Methods
(See Items 8 and 9 under Introduction)

Petitioner's Remarks

"The residues of ETU will be determined by Method TR36F-82-15 supplied by the Sponsor (Appendix A).

Maneb residues will be determined by HLA method entitled "A Gas-Chromatographic Method for Measurements of Dithane" (Appendix B)."

Our Comments

No objection to the overall principle of both methods. RCB recommends, however, that all tissue samples be stored frozen in a sliced or diced condition rather than a homogenized state. Samples can be homogenized just prior to extraction for ETU analysis or sample pieces directly added to the digestion flask at time of mane b analysis. These analytical procedures must be properly validated via recovery runs with each test substance at each sampling interval.

G. Study Reporting Requirements

Petitioner's Remarks

"This report will contain, but not be limited to, the following:

- o A description of the methods and procedures used for the determination of the stability of ETU residues
- o The results of samples analyzed, expressed in ng/g (ppb)
- o Recovery and control values for each analytical run
- o A graphic display that shows results obtained versus time of storage for each matrix

A draft final report will be issued no later than 30 days after the analysis is completed, and a final report no later than 14 days after receiving comments from the Sponsor.

This study will be conducted in accordance with Environmental Protection Agency (EPA) Pesticide Program Good Laboratory Practice Standards (40 CFR 160). The Quality Assurance Unit of HLA will review the study and the final report.

Original data, or copies thereof, will be available at HLA to facilitate auditing the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data generated by HLA, as well as the final report, will be retained in the HLA archives for the period specified by the EPA Good Laboratory Practice Standards."

Our Comments

It appears that the petitioner is making adequate provision for recordkeeping in conjunction with the proposed study.

V. Draft Protocol for Maneb Stability in Animal Commodities

A. Study Objective

Petitioner's Remarks

"The objective of this study is to determine the stability of Maneb in milk, eggs, and tissue samples stored frozen in screw-cap bottles. This study is in support of HLA Study No. 6181-104 entitled "Study of Residues of Maneb and its Primary Metabolite in Feces, Tissues, and Eggs of Laying Hens" and HLA Study No. 6181-105 entitled "Study of Residues of Maneb and Primary Metabolites in Urine, Feces, Tissues, and Milk of Dairy Cattle."

Our Comments

The objective of this study (to validate maneb residues only in animal commodities derived from previously submitted dairy cattle and poultry feeding studies) does not satisfy the requirements delineated in the March 31, 1987 DCI Notice for maneb. (See RCB's reference to the DCI Notice under IV.A. above.) Maneb and ETU storage stability data are required for repeated animal feeding studies.

B. Test Matrix

Petitioner's Remarks

"This study will be conducted on whole eggs, milk, muscle, liver, kidney, and fat of poultry and dairy cattle."

Our Comments

No objections.

C. Test Material

Petitioner's Remarks

"Compound:	Maneb [[1,2 ethanediylbis [carbamodithioate]](2-) manganese
Lot No.:	1A5026
Molecular formula:	C ₄ H ₆ MnN ₂ S ₄
Molecular weight:	265.29
Physical state:	Solid"

Our Comments

ETU must be included as test material in the final protocol. Is it the petitioner's intent to include ETU in the final protocol contingent upon the results obtained with ETU fortifications in the draft pilot study protocol described above?

D. Sample Amendment

Petitioner's Remarks

"Before the control samples are amended with Maneb, the spiking mixture will be analyzed by HLA to verify the amount to be added to each sample.

Samples will be amended with the test material at the 500-ppb level; kidney samples will be at 1.0 ppm. The samples will be blended, and a 10.0 g portion will be weighted directly into a 20- to 50- mL screw-cap bottle storage. Milk and egg samples and their controls will be stored in polypropylene bottles, while the tissue samples and their controls will [be] stored in glass bottles with screw caps, to simulate storage as for HLA Study Nos. 6181-104 and 6181-105. The individual 10 g samples will be spiked frozen with 10 to 25 μ L of an appropriate level of suspended Maneb in H₂O solution to correspond to 5.0 μ g of Maneb (500 ppb). The samples, including controls, will be stored frozen at approximately -20°C in accordance with HLA SOP OP-GENC 21 until analysis. Each sample will be assigned a unique HLA number for identification."

Our Comments

See RCB's comments (to include fortification of samples with ETU) under IV.D. above. In addition, the number of samples to be amended must be specified.

E. Sampling Intervals/Sampling

Petitioner's Remarks

"After samples are amended, they will be analyzed at 0, 7, 14, 30, 45 \pm 2, 60 \pm 2, 90 \pm 2, 120 \pm 2, and 180 \pm 2 days after storage. The Day 0 samples will be analyzed without being frozen. Two amended samples, one control, and one control spiked at 500 ppb Maneb will be analyzed for each time period for each matrix."

Our Comments

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

F. Analytical Methods

(See Items 8 and 9 under Introduction.)

Petitioner's Remarks

"Maneb residues will be determined by HLA method "A Gas Chromatographic Method for Measurement of Dithane" (Appendix A)."

Our Comments

See RCB's comments under IV.F. above. In addition, validated ETU methodology must be included in final storage stability protocol for animal commodities.

G. Study Reporting Requirements

Petitioner's Remarks

"The report will contain, but not be limited to, the following:

- o A description of the methods and procedures used for the determination of the stability of maneb residues
- o The results of samples analyzed, expressed in ng/g (ppb)

- o Recovery and control values for each analytical run
- o A graphic display that shows results obtained versus time of storage for each matrix

After the 60-day sampling interval, an interim report will be issued that details the results obtained up to that point for each matrix. A draft final report will be issued no later than 30 days after the analysis is completed, and a final report no later than 14 days after receiving comments from the Sponsor.

This study will be conducted in accordance with Environmental Protection Agency (EPA) Pesticide Program Good Laboratory Practice Standards (40 CFR 160). The Quality Assurance Unit of HLA will review the study and the final report.

Original data, or copies thereof, will be available at HLA to facilitate auditing the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data generated by HLA, as well as the final report, will be retained in the HLA archives for the period specified by the EPA Good Laboratory Practice Standards."

Our Comments

Reporting requirements for both maneb and ETU must be provided. See RCB's comments under IV.G. above.

Recommendations

1. 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.

2. Final protocols should not be designed to validate previously submitted residue, processed commodity, or animal commodity studies for the reasons given throughout this review. The final protocols submitted to the Agency must reflect storage

stability studies run concurrently with repeated crop residue, processing, and animal feeding studies.

3. Since the Agency has decided not to accept storage stability studies designed to validate existing crop residue and animal feeding studies, information provided by the petitioner on "chain of custody data" will not be required. However, if the registrant attempts to validate any of the existing data, it would be at his own risk. Therefore, RCB's review of this protocol is directed toward storage stability studies designed to validate new data in keeping with the 3(c)2(B) DCI Notice of 3/31/87.

cc: W.Boodee, E.Zager, Reviewer-M.Kovacs, S.F., R.F., Ellenberger-
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