PMSD/15A

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

SEP 8 1988

September 8, 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Mancozeb (014504) Storage Stability Protocols
Rohm and Haas Response to Registration Standard
Rohm and Haas letter of 1/5/88
EPA Reg. No. 707-78
[No MRID No., RCB No. 3202]

FROM: Susan V. Hummel, Chemist
Special Registration Section II
Dietary Exposure Branch
Health Effects Division (TS-769C)

Susan V. Hummel

THRU: Edward Zager, Section Head
Special Registration Section II
Dietary Exposure Branch
Health Effects Division (TS-769C)

E Zager

TO: Lois Rossi, PM #21
Herbicide Fungicide Branch
Registration Division (TS-767C)

Rohm and Haas has submitted a response to the Mancozeb Registration Standard, consisting of protocols for storage stability studies for crops and animal products.

Mancozeb is a coordination product of zinc ion and maneb (manganese zinc ethylenebisdithiocarbamate). Tolerances have been established for residues of mancozeb on a number of raw agricultural commodities including kidney and liver at 0.5 ppm (40 CFR 180.176). Tolerances for mancozeb are calculated as zineb equivalents. An interim tolerance for residues of mancozeb in potatoes is found in 40 CFR 180.319. Food and feed additive tolerances have been established for several processed commodities (21 CFR 193.460 and 21 CFR 561.410). One tolerance petition for residues of mancozeb in lettuce, peppers, and beans is in reject status (PP#3F2949, M. Kovacs, 12/10/87, RCB No. 2654).

The Product and Residue Chemistry chapters for the Mancozeb Registration Standard were completed on 9/10/86. An update was completed on 1/27/87. The Mancozeb Registration Standard (Guidance Package) was issued in April, 1987. A Special Review of the ethylene bisdithiocarbamate pesticides (EBDCs), including mancozeb has been initiated. (52 FR 27172, 7/17/87).

CONCLUSIONS

1. Because of the differences in the results obtained by the various EBDC registrants, we have reconsidered our previous conclusion regarding storage stability data. We will require storage stability studies for EBDC's and ETU conducted concurrently with residue analyses for each crop group, for each growing season, and for each laboratory conducting residue studies.

Storage stability samples fortified with mancozeb must be analyzed for mancozeb and ETU. Storage stability samples fortified with ETU must be analyzed for ETU. The Registration Standard deficiency for storage stability data is outstanding. Because of differences between laboratories, we will not accept storage stability data from one laboratory to support residue data from another laboratory.

2. Comments on Storage Stability Protocols

a. Test Materials and Spiking Solution: The Registration Standard specifically requires that the study be conducted using the pure active ingredient (PAI), not a formulated product (Dithane M-45).

b. Testing Facility: The use of Enviro-Bio-Tech to conduct the storage stability study is acceptable, provided that Enviro-Bio-Tech also conducts all analyses of crop field trial samples and animal feeding study samples. We will not accept storage stability data from one laboratory to support residue data from another laboratory.

c. Samples to be analyzed: The samples to be analyzed should be one sample from each crop group for which mancozeb is registered. The choice of samples for animal commodities are acceptable.

d. Preparation and analysis of crop samples: The storage stability samples should be handled in exactly the same manner as the field trial or feeding study samples. The use of whole crop samples is acceptable, provided this is how the crop field trial samples are handled. The registrant should be cautioned that it is extremely difficult to uniformly spike whole samples. The partial thawing, homogenizing, and refreezing of storage stability samples 24 hours before analysis would also be acceptable, provided this is how the field trial samples are handled.

3. The registrant should also consult the OPP Position Document on Storage Stability for further information.

RECOMMENDATIONS

We recommend that the registrant be informed that storage stability data are required to support all crop field trial studies and animal feeding studies and that the registrant be advised to submit the required data. We recommend that this memo including our comments regarding the submitted protocol be forwarded to the registrant. We recommend that the OPP Position Document on Storage Stability, which is attached to this memo, be forwarded to the Registrant.

## Detailed Considerations

### Registration Standard Deficiency 5 - Storage Stability

Available storage stability data are adequate to demonstrate that mancozeb is stable in/on frozen plant samples for up to 12 months and ETU is stable for up to 6 months in frozen plant samples.

To support crop residue data, storage stability studies must be conducted on both weathered samples (mancozeb) and fortified frozen samples (mancozeb, metabolites and ETU) of one representative crop from each crop grouping (40 CFR 180.34) on which registered uses of mancozeb exist. Analyses of each crop must be conducted over a time period that includes the time interval that the raw agricultural commodity is held in frozen storage prior to the crop residue analysis. To support residue data on processed commodities, fortified storage stability data are required for all processing studies submitted to the Agency. Analyses must be conducted over a time period that includes the frozen storage of the raw agricultural commodity prior to processing and each processed commodity prior to the residue analysis. Protocols for these studies must be submitted to and approved by the Agency prior to initiating the studies.

(a) Storage stability data using weathered samples. Data are required on the parent compound, mancozeb, in which crop samples field treated with a typical end use product are frozen immediately upon harvesting. The integrity of the samples must be maintained by freezing. The samples must be analyzed for mancozeb on the day they arrive at the analytical laboratory, and then stored frozen and analyzed periodically for mancozeb during the time intervals specified in the Agency approved protocol.

(b) Storage stability data using fortified samples. Data are required on mancozeb, ETU, and metabolites in which a group of untreated samples of raw agricultural commodities and processed crops are fortified (spiked) with only mancozeb pure active ingredient, another group of samples is fortified with only ETU, and other groups are fortified individually with each additional metabolite. Immediately after fortification, the samples fortified with mancozeb must be analyzed for mancozeb and ETU; samples fortified with ETU must be analyzed for only ETU; and samples fortified with other metabolites must be analyzed for only the metabolite with which the sample was fortified. Sample integrity must be maintained by freezing, and analyses for mancozeb, ETU, and metabolites must be conducted periodically during the time intervals specified in the Agency approved protocol.

(c) Storage stability data for livestock/poultry feeding studies. If cattle and poultry feeding studies are required (see Registration Standard Guidance Package, Data Table footnotes 71 and 72), fortified storage stability studies will be required on all animal commodities (i.e., tissues, milk and eggs) for which residue data are submitted to the Agency. Analyses must be conducted over a time period that includes the time interval that each commodity is held in frozen storage prior to residue analyses.

(These deficiencies were in the text and not in the Guidance Package Table Footnotes.)

All requested residue data must be accompanied by data regarding storage intervals and conditions of sample storage from harvest until analysis.

If metabolism studies reveal the presence of other metabolites of concern, then storage stability studies must be conducted on these additional metabolites for the length of time the samples were stored.

Previous RCB Comment (S. Hummel memo of 4/21/88)

The registrant is correct in stating that the fortified storage stability studies they have submitted are adequate to show that mancozeb is stable in frozen storage for up to 12 months and that ETU is stable in frozen storage for up to 6 months. However, storage intervals and conditions of sample storage from harvest until analysis were not available for the residue data reviewed for the Registration Standard. Thus, any

data submitted for which the frozen storage interval is longer than 12 or 6 months for mancozeb or ETU, respectively, are not valid.

Weathered storage stability studies will not be required if all samples were analyzed within 12 months of harvest for mancozeb and within 6 months for ETU, and were stored frozen from harvest until analysis. If any samples were stored longer than 12 months and 6 months, then both weathered and fortified storage stability data are needed.

Current RCB Comment

Differing results were obtained by the various registrants of the EBDC fungicides for the stability of ETU (and the various EBDC's) in frozen storage. It is obvious that ETU stability is dependent upon many factors, including the identity of the commodity, storage conditions, and sample handling, including analysis and analyst familiarity with the analytical methodology. We believe that the storage stability data submitted by each registrant reasonably reflect the storage conditions and sample handling done by that registrant (or contract laboratory conducting the study) at the time the storage stability study was conducted.

Because of the differences in the results obtained by the various EBDC registrants, we have reconsidered our previous conclusion regarding storage stability data. We will require storage stability studies for EBDC's and ETU conducted concurrently with residue analyses for each crop group, for each growing season, and for each laboratory conducting residue studies.

Storage stability samples fortified with mancozeb must be analyzed for mancozeb and ETU. Storage stability samples fortified with ETU must be analyzed for ETU. The Registration Standard deficiency for storage stability data is outstanding. Because of differences between laboratories, we will not accept storage stability data from one laboratory to support residue data from another laboratory.

Storage Stability Protocol

Rohm and Haas Protocol No. 31P-87-15, "Sample Storage Stability Study: Stability of ETU and Mancozeb Residues in Selected crops and Animal Tissues."

Objective: To determine the stability of mancozeb and ETU residues during long-term cold storage at -18 to -20 C.

Testing Facility: Enviro-Bio-Tech, Bernville, PA

Test Materials: Mancozeb as Dithane M-45, ETU (lot Numbers given), solvents, HPLC or Pesticide grade, other reagents ACS grade or purer.

Samples to be analyzed: apples, lettuce, tomatoes; cow liver, fat, kidney, muscle, milk, and thyroid; hen liver, kidney, fat, muscle, gizzard, eggs.

Spiking Solution: A 1000 ppm suspension of mancozeb (as Dithane M-45) in acetone; and a 100 ppm solution of ETU will be used. The mancozeb suspension will be stirred continuously throughout the spiking procedure.

Preparation of Crop Samples: In order to simulate actual practice, whole apples, tomatoes, and spinach will be spiked and stored frozen. Immediately prior to analysis, the samples will be homogenized.

Apples and tomatoes and lettuce leaves will be arranged in 10 rows and 10 columns on a foil lined tray. The spiking solution will be sprayed uniformly over the exposed surfaces, allowed to dry, the samples turned over, and the unsprayed side will be sprayed with additional spiking solution. After drying, the samples will be placed in polyethylene bags and stored in a freezer at -18 to -20 C. Samples will be subsampled according to a diagram given in the protocol.

Tissue samples will be homogenized and frozen for 24 hours (-18C) in individual vials. Twenty vials will be removed and spiked with freshly prepared mancozeb spiking solution. Two vials will be analyzed as day 0 samples, and the rest immediately returned to the freezer. Twenty vials will be removed and spiked with freshly prepared ETU spiking solution. Two vials will be analyzed as day 0 samples, and the rest immediately returned to the freezer.

Milk will be homogenized, subsampled, placed in vials, and frozen for at least 18 hours. Spiking will be done as indicated above for tissue samples, except that the acetone from the mancozeb spiking solution will be allowed to evaporate before returning the mancozeb spiked samples to the freezer.

Egg whites and yolks will be homogenized, subsampled, placed in vials, and frozen for at least 18 hours. Spiking will be done as indicated above for tissue samples, except that the acetone from the mancozeb spiking solution will be allowed to evaporate before returning the mancozeb spiked samples to the freezer.

Sampling Intervals: Crop samples will be analyzed at 0, 1/2, 1, 2, 4, 6, 9, 12, 15, and 18 months. Animal tissues will be

analyzed at 0, 1/2, 1, 2, 4, and 6 months.

Analysis of samples:

Crop samples: The day before each sampling date, the appropriate subsample will be removed from cold storage along with one control sample. The samples will be allowed to thaw for 10 minutes at room temperature before being homogenized in a food processor in the presence of dry ice. The homogenized sample will be returned to the freezer for 24 hours to allow the dry ice to sublime. On the day of analysis, the homogenized sample will be removed from the freezer. Duplicate aliquots of the treated sample will be removed and analyzed for the appropriate residue. Duplicate untreated samples will be removed from the homogenate. One untreated aliquot will serve as a negative control while the other will be spiked with an appropriate amount of freshly prepared spiking solution. This fresh spike should be prepared so that the residue level will approximate that found in the crop samples.

Tissue samples: At each sampling date, two treated vials will be removed for each analyte along with four untreated tissue samples. One control vial will be spiked with an appropriate amount of spiking solution for each analyte. The remaining two control sample will serve as negative controls.

Prior to analysis, all samples should be thawed, but should not be permitted to stand at room temperature for more than 60 minutes.

ETU analysis is by the method of Onley, J. Assoc. Offic. Analyt. Chem., 60 (1977), 1105-1110. Each sample will be extracted and applied to the clean-up column before extracting the next sample. In this way, time between extraction and isolation of the ETU fraction will be minimized. The remainder of the procedure should be followed as reported in the above reference.

EBDC analysis is by the standard $CS_2$ procedure as described by Pease and modified by Keppel, J. Assoc. Offic. Analyt. Chem., 54, (1971), 528-532. (colorimetric method); or the modified method described in Rohm and Haas Technical Report 36F-82-20 (GC method). Once the sample has been thawed properly, it should be placed in the digestion flask and the vial should be rinsed with an appropriate amount of 10% $Na_4EDTA$. The EDTA rinse should also be added to the digestion flask. The remainder of the procedure should be followed as reported in the above reference.

Reporting: An interim report for crop storage stability is expected at 6, 12, and 18 months; and for tissue storage stability at 3 months. At the conclusion of the study, all original chromatograms and data should be forwarded to the sponsor.

7

Comments

Test Materials and Spiking Solution: The Registration Standard specifically requires that the study be conducted using the pure active ingredient (PAI), not a formulated product (Dithane M-45).

Testing Facility: The use of Enviro-Bio-Tech to conduct the storage stability study is acceptable, provided that Enviro-Bio-Tech also conducts all analyses of crop field trial samples and animal feeding study samples. We will not accept storage stability data from one laboratory to support residue data from another laboratory.

Samples to be analyzed: The samples to be analyzed should be one sample from each crop group for which mancozeb is registered. The choice of samples for animal commodities are acceptable.

Preparation and analysis of crop samples: The storage stability samples should be handled in exactly the same manner as the field trial or feeding study samples. The use of whole crop samples is acceptable, provided this is how the crop field trial samples are handled. The registrant should be cautioned that it is extremely difficult to uniformly spike whole samples. The partial thawing, homogenizing, and refreezing of storage stability samples 24 hours before analysis would also be acceptable, provided this is how the field trial samples are handled.

The registrant should also consult the OPP Position Document on Storage Stability for further information.

Attachment: OPP Position Document on Storage Stability
[to be forwarded to registrant]

cc:R.F., circu, S. Hummel, mancozeb S.R.F. (Hummel), mancozeb R.S.F. (Boodee), mancozeb S.F., V. Bael (SRB/RD), TOX, PMSD/ISB
RDI:EZ:09/08/88:RDS:09/08/88
TS-769:RCB:SVH:svh:RM810:CM#2:x77324:09/08/88

A POSITION DOCUMENT OF THE U.S. ENVIRONMENTAL PROTECTION AGENCY

OFFICE OF PESTICIDE PROGRAMS

# EFFECTS OF STORAGE (STORAGE STABILITY) ON VALIDITY OF PESTICIDE RESIDUE DATA

**Hazard Evaluation Division**
**Residue Chemistry Branch**

**August 1987**

# EFFECTS OF STORAGE (STORAGE STABILITY) ON VALIDITY OF PESTICIDE RESIDUE DATA

## PURPOSE:

The purpose of this document is to alert all participants in the federal pesticide registration and tolerance process of the need to provide adequate data on the possible effects of storage on the magnitude of the residue in commodities during the time between sample collection and sample analysis. This policy applies to all samples derived from supervised field trials, metabolism and feeding studies; processing studies; and to all magnitude of the residue data submitted to EPA. Lack of adequate storage stability data has become a major factor in delaying registrations of pesticides. New chemicals that are subjected to the EPA screening for acceptability for review will be checked for adequacy of storage stability data.

This document does not introduce any new data requirements or revisions into the Pesticide Assessment Guidelines-Subdivision O (§171-4). It clarifies ambiguities in interpretation of those existing Guidelines and thereby should aid registrants in submitting acceptable data packages on the magnitude of the residue to facilitate the Agency's review process.

## BACKGROUND:

Data on the magnitude of the residue for crops, meat, milk, poultry, eggs, fish, and processed commodities are required by 40 CFR §158.125 to support the registration of any pesticide intended for use on a food or feed crop under the amended Federal Insecticide, Fungicide, and Rodenticide Act. These data are also needed to estimate the exposure of the general population to pesticide residues in food, and for establishing tolerances for pesticide residues in or on raw and processed foods or feeds under provisions of Sections 408 and 409, respectively, of the Federal Food, Drug, and Cosmetic Act.

In most cases, treated samples undergo a period of storage (usually frozen) prior to their analyses for pesticide residues. During this time it is possible that residue levels could decline in the matrices of interest - that portion of the plant and/or animal commodities on which pesticide tolerances/exemptions are sought. Therefore, unless samples are quickly analyzed, data will need to be provided by the petitioner/registrant to validate the magnitude of residue levels of pesticides in commodity matrices during such a storage period.

EXPERIMENTAL DESIGN AND TEST PROCEDURES:

The storage stability study should be experimentally designed to answer the following question: Are the parent pesticide and its metabolite(s) that together comprise the "total toxic residue" which is to be regulated stable in the "matrices of interest" during storage? The term "total toxic residue" is used to describe the sum of the parent pesticide and its degradation products, metabolites (free or bound), and impurities that are considered to be of toxicological significance, and therefore warrant regulation.

For unrelated commodities in petition submissions, the petitioner will need to supply supporting storage stability data for the total toxic residue (i.e., for each component specifically expressed in the tolerance) for each commodity. If the analytical method is capable of quantitating each component of the residue individually, then a spiking mixture may be used for fortification purposes. If the analytical method is only capable of measuring the total residue, then the storage stability of each component comprising the total toxic residue is needed.

Storage stability studies with periodic sample analysis should normally be run concurrently with the storage of treated samples. However, in certain situations where pesticides are prone to breakdown or have high volatility, it is advisable to run a storage stability study in advance of the magnitude of the residue study so that proper storage and maximum time of storage can be determined before treated samples are collected and stored. In these cases, storage stability studies should also be run concurrently with the storage of treated samples.

Samples used in storage stability studies should be stored exactly like the field incurred residue samples; e.g., in the same freezer, in the same types of containers, and for the same lengths of time. Deviations from this may result in the treated samples being considered invalid due to lack of appropriate supporting storage stability data.

The use of unblended samples, blended samples, or raw sample extract for a storage stability study is acceptable, provided the supervised trial samples to be used to determine the magnitude of the residue are handled in a similar manner. If samples are stored in more than one of these conditions (ie, some samples blended prior to storage and some samples stored whole), then storage stability data are needed to validate each condition of treated sample storage.

The registrant or petitioner may choose whether to use field incurred residue samples of known value analyzed prior to storage, or to use fortification samples in the storage stability study.

It is always advisable to have extra storage stability samples available to allow for unforeseen delays in analysis, and to verify the results of check sample analysis should it be necessary to reanalyze them for possibly aberrant results.

If limited decline of the residue is shown to occur during the storage period, correction factors may be applied to the supervised trial residue results to determine the appropriate level at which the proposed tolerance should be established. If extensive decline of the residue is shown to occur, storage of supervised trial samples prior to analysis should be avoided. Unless storage stability has been documented previously, the Agency suggests samples be analyzed as soon as possible or within 14 days of collection to avoid storage stability problems. These studies are particularly important when the residue is labile or volatile. For those compounds known to be labile or volatile, storage stability data are even more important. These samples should be analyzed as soon as possible (within several days of collection).

RESIDUE RESULTS:

The petitioner/registrant's report on storage stability studies should include all information necessary to provide a complete and accurate description of the commodities that were stored (whether raw or processed); the test compound(s); the experimental design and storage conditions (e.g., freezer temperature, length of storage, type of containers, etc.); residue methods(s) and instrumentation; storage stability results and reporting of the data; statistical analysis; and quality control measures/precautions taken to ensure the validity of these operations, including the dates for each step above.

After a series of appropriate storage stability studies on unrelated commodities have been submitted that show similar results, future petitions on related commodities can reference previously accepted studies in lieu of conducting additional storage stability studies. Translating a storage stability study from one commodity to another will be considered appropriate only if both commodities are related (e.g., in the same crop group), and if the experimental design is considered appropriate to current considerations.

FOR FURTHER INFORMATION:

The reader is referred to the Agency document, "Pesticide Assessment Guidelines, Subdivision O, Residue Chemistry, Series 171-4, Storage Stability Study, Addendum on Data Reporting", which is available from the National Technical Information Service, Springfield, VA [NTIS Document #PB86-248192], for additional details about conducting a storage stability study.

If you have questions regarding this document, the EPA contact is Francis D. Griffith, Jr., Hazard Evaluation Division (TS-769C), Office of Pesticide Programs, Environmental Protection Agency, 401 M St., SW, Washington, D.C. 20460, telephone (703) 557-7484.

Edwin F. Tinsworth, Director
Registration Division