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

JUN 8 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: ID No. 464-5111 1,3-Dichloropropene (Telone) - Amended Protocol for (Lettuce/Spinach) and Soybean Plant Metabolism Studies (No Accession Number); (DEB No. 5109)

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TO: Herman T. Toma, PM Team 21
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and

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THRU: Richard D. Schmitt, Ph.D., Acting Chief
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Introduction

In the current submission, dated February 23, 1989, Dow Chemical U.S.A. has provided the Agency with amended (lettuce/spinach) and soybean plant metabolism study protocols for ^{14}C-1,3-Dichloropropene (Telone). The original plant metabolism protocols were previously submitted by the petitioner and evaluated in DEB's M. F. Kovacs, Jr., 7/7/88 memorandum re: ID No. 464-511-1,3, Dichloropropene (Telone) - Response to Registration Standard Data Call-In - (Lettuce/Spinach) and Soybean Plant Metabolism Studies (MRID Nos. 405718-00 through 405718-02) [RCB No. 3654]. The amended protocols were submitted by the registrant in response to deficiencies cited in the aforementioned memorandum and further discussed at a meeting between EPA and Dow Chemical USA representatives on 12/9/88.

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Summary of Deficiencies That Still Need Resolution

All of the Deficiencies/Recommendations cited in DEB's M. F. Kovacs, Jr., 7/7/88 memorandum remain outstanding (i.e.,):

- The qualitative nature of the total terminal residue in lettuce, spinach and soybeans has not been adequately characterized and therefore the plant metabolism studies should be repeated.
- The new plant metabolism studies should be suported by storage stability data.
- New metabolism protocols should be resubmitted which include (1) storage stability considerations, (2) identity of radiolabeled impurities arising from the test material, (3) inclusion of 1,2-dichloropropane as a test substance and (4) delineation of specific procedures to further fractionate and characterize/identify ^{14}C components in all soluble and insoluble extracts/fractions.

Conclusions

A. Conclusions outlined in DEB's M. F. Kovacs, Jr., 7/7/88 review, relating to the qualitative nature of the terminal residue in lettuce, spinach and soybeans, the submission of new plant metabolism studies addressing the identity of radiolabeled impurities arising from the test material and including 1,2-dichloropropane as the test substance were not addressed in the present submission. Conclusions related to the submission of new metabolism protocols addressing the delineation of specific procedures to further fractionate and characterize/identify ^{14}C components in all soluble and insoluble extracts/fractions and storage stability considerations for the ^{14}C components are partially addressed in the present amended protocol submission.

B. The deficiencies (conclusions) with regard to the present amended (lettuce/spinach) and soybean protocol submission are as follows:

1. Storage Stability Data for New Protocols

The registrants' postulated analytical equivalency between reanalyzed (1989) and original (1987) frozen ^{14}C samples obtained from the lettuce/spinach and soybean metabolism studies does not establish the integrity of residues in frozen and stored samples,

especially since no residues were detected in 1987. The frozen storage stability or integrity of ^{14}C samples must be established for the time interval encompassing initial collection or harvesting (T_1) to time of initial ^{14}C characterization or analysis (T_2), not for the time interval encompassing initial analysis (T_2) to some pre-determined future time, in this case 2 years (T_3).

Therefore, that portion of Recommendation 3 of the M. F. Kovacs, Jr., 7/7/88 review which states that in new metabolism protocols "all ^{14}C samples should be analyzed within 2 weeks of harvest and accompanied by a storage stability study for the parent compound plus all anticipated metabolites if samples are not analyzed within this time interval" remains outstanding.

2. Specific Procedures to Fractionate and Characterize/ Identify ^{14}C Components

The registrant has not delineated specific procedures to further fractionate and characterize/identify ^{14}C components in all insoluble fractions.

Therefore, that portion of Recommendation 3 of the M. F. Kovacs, Jr., 7/7/88 review which states "delineation of specific procedures to further fractionate and characterize/identify ^{14}C components on all insoluble extracts/fractions" remain outstanding.

3. For the reasons given in Conclusion 1 above, the registrant is advised by DEB that reanalysis or additional ^{14}C characterization of stored reserve samples obtained from the 1987 lettuce/spinach and soybean metabolism studies is unacceptable and will not satisfy DEB's outstanding requirement for the submission of new plant metabolism studies (see Recommendation 2 of DEB's M. F. Kovacs, Jr., 7/7/88 review).

Recommendations

1. DEB recommends that a copy of this review be sent to the registrant.

2. DEB recommends that the deficiencies cited under "Summary of Deficiencies that Still Need Resolution" be addressed by the registrant.

3. Revised protocols should be developed, submitted by the registrant and approved by DEB before any additional plant metabolism studies are undertaken. The revised

protocols must address all of the protocol deficiencies stated in previous reviews and cited in this review under Conclusions 1 through 3.

Detailed Considerations

The registrant, Dow Chemical U.S.A. has included the following amended protocols in its February 23, 1989 letter of submittal to Herman T. Toma, PM Team (21), RD/EPA:

1. A revised protocol dated February 21, 1989, titled: A metabolism study of lettuce and spinach grown in soil treated with ^{14}C-dichloropropenes (Protocol No. 56-87, Amendment No, 6).

2. A revised protocol dated February 21, 1989, titled: A metabolism study of soybeans grown in soil treated with ^{14}C-dichloropropenes (Protocol No. 55-87, Amendment No. 6).

Deficiencies/recommendations which were discussed in DEB's July 7, 1988 review (M. Kovacs) are restated below, followed by the registrant's responses and DEB's conclusions.

Recommendation 3. (M. F. Kovacs, Jr., 7/7/88 review)

3. Prior to conduct and resubmission to RCB of additional ^{14}C lettuce and soybean metabolism studies, the registrant should submit to RCB for our evaluation new metabolism protocols addressing the concerns raised by RCB throughout this review and enumerated in the Other Considerations section of this review. These are:...all ^{14}C samples should be analyzed within 2 weeks of harvest and accompanied by a storage stability study for the parent compound plus all anticipated metabolites if samples are not analyzed within this time interval....and (3) delineation of specific procedures to further fractionate and characterize/identify ^{14}C components in all soluble and insoluble extracts/fractions.

   In conjunction with the conduct of the resubmitted plant metabolism studies, the registrant is directed to the Pesticide Assessment Guidelines Subdivision O Addendum 3 on Data Reporting, Nature of the Residue: Plants for general guidance in this area, a copy of which is attached to this review.

Registrant's Response to Recommendation 3

Storage Stability Data for New Protocols

Study Title: A metabolism study of lettuce and spinach grown in soil treated with ^{14}C-dichloropropenes.

Original Protocol Section:

"...extracts will be examined by radio-HPLC and GC for the presence of 1,3-dichloropropene, 3-chloro-2-propen-1-ol and other metabolic products containing ^{14}C. Direct metabolites of ^{14}C-dichloropropene present at or above 0.1 ppm will be isolated and characterized or identified.

Amended Protocol Section:

1) Re-analysis of the 1987 tissues from frozen storage was competed during February 1989. The results of these analyses showed a close correspondence with those reported in GHC-2031, both qualitatively and quantitatively. Therefore, the data showed that the nature of the residues present did not change significantly during the storage period, and that the tissues remain valid for further analyses.

Study Title: A metabolism study of soybeans grown in soil treated with ^{14}C-dichloropropenes

Paragraph 1 the same as above for lettuce and spinach metabolism study.

Amended Protocol Section:

i) the same as above for lettuce and spinach metabolism study except substitute "GHC-2032" for "GHC-2031".

DEB's Comments/Conclusions re: Storage Stability Data for New Protocols (Amended Protocol Section i.)

DEB does not consider the 1989 reanalysis of stored and frozen GHC-2031 (lettuce and spinach) or GHC-2032 (soybean) ^{14}C samples obtained from the original 1987 metabolism studies as demonstrating the validity (or integrity) of these samples for further analysis as proposed in paragraph ii of the amended protocols.

The storage stability or integrity of these ^{14}C samples was questioned for the time interval encompassing initial collection or harvesting (T_1) to time of initial ^{14}C characterization or analysis (T_2) not for the time interval encompassing initial analysis (T_2) to some future time, in this case, 2 years (T_3).

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Analytical equivalency between T_2 and T_3 analyses does not establish sample integrity for unstable ^{14}C components such as 1,3-dichloropropene, especially in a case such as this, where no residues were detected at the original analysis date (T_2). Based on the time interval that the original 1987 ^{14}C samples were stored prior to analysis (lettuce and soybeans up to 28 weeks and spinach up to 12 weeks) and furthermore based on storage stability data for 1,3-dichloropropene in lettuce and potatoes as presented in the Residue Chemistry Chapter of the Telone Registration Standard (i.e., after 75 days 55 to 66% and 27 to 40% of the initial level remained in/on lettuce and potatoes respectively) it is not surprising that the parent compound 1,3-dichloropropene including its anticipated metabolites were non-detectable in the initial analysis of the 1987 samples, let alone when these same samples were reanalyzed 2 years later.

Therefore, DEB reiterates its recommendations made in the D. Edwards, 11/5/86 Telone protocol review, and restated in Recommendation 3 of the M. F. Kovacs, Jr., 7/7/88 review, that in future ^{14}C metabolism studies, ^{14}C samples be analyzed <u>within</u> 2 weeks of harvest and further that frozen control samples fortified with Telone and anticipated metabolites be maintained and analyzed along with field treated samples to determine the degree of loss of residues during storage if samples are not analyzed within 2 weeks.

Recommendation 3 of the M. F. Kovacs, Jr., 7/7/88 review as it relates to storage stability considerations for ^{14}C components of resubmitted metabolism studies remains outstanding.

<u>Registrant's Response to Recommendation 3</u>

<u>Procedures to Further Fractionate and Characterize/Identify ^{14}C Components</u>

<u>Original Protocol Section</u>:

> "...extracts will be examined by radio-HPLC and GC for the presence of 1,3-dichloropropene, 3-chloro-2-propen-1-ol and other metabolic products containing ^{14}C. Direct metabolites of ^{14}C-dichloropropene present at or above 0.1 ppm will be isolated and characterized or identified."

<u>Amended Protocol Section</u>:

> ii) Having established that the nature of the ^{14}C-residues was unchanged during frozen storage, further appropriate characterization methods will be employed. In view of the data obtained in previous dichloropropene metabolism studies, supporting natural incorporation of ^{14}C from ^{14}C-dichloropropene, the following methodology is considered most appropriate: a) solubilization of

^{14}C-residues using aqueous alcohol mixtures, such as 50% methanol, b) separation of the solubilized ^{14}C-residues into anionic, cationic and neutral fractions (i.e., organic acids, amino acids and pigments + sugars + macromolecules, respectively) using ion exchange resins. c) Further separation of these fractions according to their organic/aqueous partition behaviour, reverse-phase HPLC and/or gel permeation chromatography, and derivative formation, as appropriate. d) Chromatographic isolation and identification of components representing 0.1 ppm, or greater, of total residue.

Rationale:

These additional investigations will provide sufficient characterization data to enable a final determination of nature of the terminal residues of ^{14}C-dichloropropene in tissues of lettuce, spinach and soybeans grown in ^{14}C-dichloropropene-treated soil.

DEB's Comments/Conclusions re: Procedures to Further Fractionate and Characterize/Identify ^{14}C Components

In the submitted amended protocol, the registrant has provided additional information on procedures to further fractionate and characterize/identify ^{14}C components in soluble extracts/ fractions which DEB recommended in its M. F. Kovacs, Jr., 7/7/88 review and was further discussed at a 12/9/88 meeting with the registrant. In that meeting, DEB recommended that the registrant consider the ^{14}C residue separation/fractionation/identification procedures for 1,3-dichloropropene residues in sugar beet roots employed by W. R. Bauriedel and L. F. Craig in their 1973 Dow Report "A Study of the Residue Present in Sugar Beet Roots Grown in Soil Treated with ^{14}C-Labeled Cis- and Trans- 1,3-Dichloropropene".

The amended protocol addresses DEB's concerns/recommendations relative to the further fractionation and characterization/identification of soluble extracts/fractions, however, it does not address that portion of DEB's Recommendation 3 in the M. F. Kovacs, Jr., 7/7/88 memorandum which requires further delineation of specific procedures to further fractionate and characterize/identify ^{14}C components in all insoluble fractions. This latter requirement was also previously addressed in DEB's D. F. Edwards 11/5/86 plant metabolism protocol review which stated "that in future ^{14}C metabolism studies the nature of unextracted residues (comprising ≥10 percent of the total recovered ^{14}C activity) should be adequately characterized following appropriate hydrolytic and fractionation procedures.

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For the reasons given above, Recommendation 3 of the M. F. Kovacs, Jr., 7/7/88 review as it relates to delineation of specific procedures to further fractionate and characterize/ identify insoluble ^{14}C components remains outstanding.

DEB advises the registrant that reanalysis or additonal ^{14}C characterization of stored reserve samples obtained from the 1987 lettuce/spinach and soybean plant metabolism studies is unacceptable and will not satisfy DEB's outstanding requirement for the submission of new plant metabolism studies (see Recommendation 2 of DEB's M. F. Kovacs, Jr. 7/7/88 review).

cc: Registration Standard File (Telone), RF, SF, E. Eldredge (PMSD/ISB), Kovacs, Circu.

RDI:D.Edwards:6/5/89:R.Loranger:6/6/89
H7509C:DEB:CM#2:Rm812A:557-7689:mb:6/7/89