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

DEC 21 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 1,3-Dichloropropene (Telone), EPA Registration No. 464-511 (No MRID Numbers) (DEB Nos. 5838 and 5919) - Evaluation of Responses to Registration Standard (Plant Metabolism)

FROM: Gary F. Otakie, P.E., Chemist
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Conclusions:

1. Significant ^{14}C residue levels (i.e., 1.32, 1.94, 2.84, 5.37, and 5.18 ppm) do occur in lettuce, spinach, soybean green forage, soybean pods and vines, and soybeans, respectively, following the proposed use of 1,3-Dichloropropene (1,3-D). Therefore, this use is definitely a food use requiring a full set of residue data requirements.

2. The nature of the total terminal residue in lettuce, spinach, and soybeans has not been adequately characterized (see Deficiencies A. 1 to 5 and B. 1 to 5 that follow in this review).

3. The registrant's hypothesis that ^{14}C residues in crop samples may be the result of natural incorporation of ^{14}C activity which probably takes place by the assimilation of the CO_2 produced by the microbial degradation of the 1,3-D in soil is not supportable based on the RCB (DEB) review of the submitted data.

4. DEB reiterates its previous recommendation made in the D.F. Edwards November 5, 1986 plant metabolism protocol review that in future ^{14}C metabolism studies sample extracts containing ^{14}C-1,3-D including its postulated metabolites should be analyzed using methods which could be used for enforcement (i.e., GC).

5. DEB reiterates its previous recommendation made in the D.F. Edwards November 5, 1986 plant metabolism protocol review that in future ^{14}C metabolism studies the nature of unextracted residues (comprising $\geq$ 10 percent of the total recovered ^{14}C activity) should be adequately characterized following appropriate hydrolytic and fractionation procedures.

6. New plant metabolism studies which include supporting storage stability data or analysis of ^{14}C samples within 2 weeks of harvest, the identity of radiolabeled impurities arising from the test material, inclusion of 1,2-D as a test substance, evaluation of the total terminal residue in plants grown in different types of mineral soils, and exhaustive procedures to further fractionate and characterize/identify ^{14}C components in all soluble and insoluble extracts/ fractions are required.

Recommendations

The soybean and lettuce/spinach plant metabolism studies submitted in response to the 1,3-Dichloropropene Registration Standard dated September 1986 are unacceptable and new plant metabolism studies are needed.

Data are required depicting the distribution and metabolism of [^{14}C] 1,3-D (including 1,2-D) in soybeans and tomatoes grown in both sandy loam and clay loam soils and harvested at regular intervals which encompass samples from both seedling and mature growth stages following a single preplant soil injection application at 10 to 12 inches below the surface in soil subsequently sealed according to label instructions (i.e., compacted) at an application rate sufficiently high to permit complete characterization of ^{14}C-residues. Planting should occur at the minimum preplant interval possible (in light of demonstrated phytotoxicity). Samples should be analyzed within 2 weeks of harvest, individual sample storage history records prepared, and frozen control samples fortified with the parent and anticipated metabolites maintained and analyzed along with field-treated samples to determine the degree of loss during storage. Samples should be analyzed to determine the total

consequently the metabolism studies were unacceptable.

5. DEB's January 6, 1989 Memorandum of Conference - December 9, 1988 of M. Kovacs included a review of the document "Summary of Status of Dichloropropene Metabolism Study" also submitted again in the current submission. Dr. Kovacs indicated during the discussion of the document that the information and conclusions provided therein by the petitioner still did not address the two main DEB concerns regarding the metabolism studies, i.e., lack of storage stability data for residues of the parent and its anticipated metabolites in the ^{14}C samples, many of which were stored longer than the 14 days maximum recommended in DEB's D. Edwards November 5, 1986 review and the lack of adequate ^{14}C characterization/identification for both solubilized moderately polar ^{14}C fractions and for insoluble conjugated and/or bound ^{14}C fractions all of which were considerably present (> 10 percent or > 0.1 ppm of TRR) in the lettuce/spinach and soybeans samples.

6. DEB's June 8, 1989 review of M. Kovacs concluded that amended protocols which included reanalysis of the 1987 tissues from the original lettuce/spinach and soybean metabolism studies were not acceptable since the storage stability sample integrity of the original samples was not demonstrated, "let alone when these samples were reanalyzed 2 years later" and the amended protocols did not address DEB's recommendations concerning "further delineation of specific procedures to further fractionate and characterize/identify ^{14}C components in all insoluble fractions." The review clearly concluded that the nature of the residue in plants was not adequately understood, all of the deficiencies cited in DEB's July 7, 1988 review of M. Kovacs remained outstanding, and that new plant metabolism protocols and new plant metabolism studies addressing all of DEB's previous recommendations were required.

7. DEB's June 19, 1989 memorandum of M. Kovacs reviewed a requested label change for Telone II involving a higher application rate than previously approved, since a more concentrated application would be made when utilizing only one chisel application per row; and concluded that since adequate plant metabolism studies were not available, DEB could not arrive at any conclusions

regarding the magnitude of all residues of concern in or on food/feed commodities from the current or proposed amended use of Telone II.

Current Submissions

The following is a summary of the current submissions from Dow Chemical U.S.A. on the plant metabolism of 1,3-D (Telone) pending review by DEB:

Letters from Robert W. Morgan

1. July 6, 1989 - Expressing surprise at receipt of DEB's June 8, 1989 review of M. Kovacs based on Dow's understanding of agreements reached in the EPA December 9, 1988 meeting; and requesting yet another meeting with Dr. Kovacs of 2 hours minimum duration to reiterate their position that the current plant metabolism studies are acceptable.

2. September 1, 1989 - Conveying a duplicate copy of the July 6, 1989 letter which had been "misplaced" and requesting expedited consideration and the arrangement of a meeting on October 4 or 5, 1989 "to thoroughly discuss this entire matter and to mutually agree on a plan of appropriate future work."

3. October 6, 1989 - Requesting to reschedule a meeting with DEB representatives originally scheduled by EPA for October 5, 1989 to October 17 or 18, since Dow had learned that their scientists involved "were not prepared to attend the meeting as scheduled."

4. October 6, 1989 - Attaching a copy of the other October 6, 1989 letter, and requesting the meeting with EPA "be arranged for October 18 (or alternatively the 17th or 20th early p.m.) to discuss various questions concerning the plant metabolism studies of 1,3-D," and enclosing a letter report of Dr. Paul Lewer concerning the storage stability of 1,3-D and 3-chloroallyl alcohol, its alleged primary metabolite in plant tissues.

Reports

1. August 24, 1989 - "1,3-D Plant Metabolism Studies," a study in which the storage stability properties of [^{14}C]-1,3-D (DCP) and [^{14}C]-chloroallyl alcohol (CAA) were investigated in separate experiments.

2. June 29, 1989 - "1,3-D (DCP) Metabolism Study" which "shows the position Dow had reached, at least as seen by them, following the December 9, 1988 visit with EPA."

3. December 15, 1989 - "^{14}C-1,3-Dichloropropene (DCP) Plant Metabolism Studies Summary of December 9, 1988 Meeting with EPA" by Paul Lewer discussing what Dow had believed Dr. Kovacs accepted, expressed, suggested and reported during the December 9, 1988 EPA meeting.

4. No Date - "Summary Status of Dichloropropene Metabolism Study" by Paul Lewer summarizing environmental parameters for DCP, plot layouts used in the lettuce/spinach/soybeans metabolism studies, application details, and residual ^{14}C activity in soil. This study was previously reviewed in DEB's January 6, 1989 Memorandum of Conference of December 9, 1988 of M. Kovacs (see Background).

Detailed Considerations

The remaining unresolved deficiencies cited in DEB's July 7, 1988 review of M. Kovacs (Response to Registration Standard Data Call-In Notice for 1,3-Dichloropropene) will be restated below followed by the petitioner's responses and DEB's comments/conclusions.

Deficiency Nos. A. 1, 2, 3, 4, and 5 and B. 1, 2, 3, 4, and 5

A. A Metabolism Study of Lettuce and Spinach Grown in Soil Treated with ^{14}C-1,3-Dichloropropenes (GH-C2031)

For the reasons given below, RCB (DEB) concludes that the submitted lettuce/spinach ^{14}C plant metabolism study does not satisfy the requirements of Section 171-4 (i.e., the qualitative nature of the total terminal residue in lettuce has not been adequately characterized/identified, and therefore the study must be repeated. DEB's comments/conclusions are enumerated below:

1. In the submitted metabolism study, significant levels (1.32 and 1.94 ppm, respectively) of total ^{14}C-residues in immature lettuce and mature spinach samples were not adequately characterized/identified by the registrant; therefore, his hypothesis that ^{14}C residues in these samples may be the result of natural incorporation of ^{14}C activity which probably takes place by the assimilation of the CO_2

produced by the microbial degradation of the 1,3-D in the soil must be further supported. It may be possible that some additional lettuce/spinach metabolism studies conducted by the registrant

utilizing ^{37}Cl-labeled 1,3-D could be helpful in this case.

2. DEB had previously suggested in its D.F. Edwards November 5, 1986 plant metabolism protocol review, and the registrant agreed in his revised March 6, 1987 protocol, to examine extracts by radio-HPLC and GC for the presence of 1,3-D and metabolites.

   In the submitted lettuce/spinach metabolism study, DEB notes that none of the potential metabolite-containing fractions (i.e., fractions identified by HPLC histograms as containing compounds of intermediate polarity) were analyzed using methods (preferably GC) which may be used for enforcement of future tolerances. DEB reiterates its previous recommendation that, in future ^{14}C metabolism studies, sample extracts containing ^{14}C-1,3-D including its postulated metabolites should be analyzed using methods which could be used for enforcement (i.e., GC).

3. DEB had previously recommended in its D.F. Edwards November 5, 1986 plant metabolism protocol review that ^{14}C samples be analyzed within 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. In response, the registrant in his March 6, 1987 revised protocol, added the following paragraph to the final protocol:

   > Additionally, control samples of lettuce will be fortified with 1,3-D and 3-chloro-2-propen-1-ol and analyzed to determine the degree of residue loss during storage. This will be a separate study, the protocol for which is being developed.

   DEB notes that this separate storage stability study cited above has not been submitted by the

registrant in conjunction with the current lettuce/spinach metabolism study. The registrant's observation in the current metabolism study, that the nature of the ^{14}C residue in various extracts and fractions (which were neither further fractionated nor characterized) did not change following 47 or 160 days in the freezer, does not satisfy DEB's earlier requirements for a storage stability study for the parent compound and its 3-chloro-2-propen-1-ol metabolite. DEB reiterates its previous recommendation that in future ^{14}C plant metabolism studies all ^{14}C samples should be analyzed <u>within</u> 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.

4. DEB had previously suggested in its D.F. Edwards November 5, 1988 plant metabolism protocol review that:

> If $\geq$ 10 percent of the total recovered ^{14}C-residues in samples from either the exaggerated (in-row) or normal use treatments remain in the tissues following extraction with a variety of polar and nonpolar solvents, attempts must be made to release and identify ^{14}C-conjugates and/or determine the nature of ^{14}C incorporated into natural plant constituents using appropriate hydrolytic and fractionation procedures.

In response to this suggestion, the registrant in his March 6, 1987 revised protocol added the following statement: "Insoluble residues will be examined for conjugate formation and for natural incorporation of the ^{14}C activity."

In the submitted lettuce/spinach metabolism study, DEB calculated that the "unextractable residue fraction" remaining following acid (1.2N HCl @ 100 °C for 4 hrs) hydrolysis of the methanol insoluble fraction contained 19.6 percent or 0.26 ppm of the total recovered ^{14}C residue from the Lettuce II sample. This

7

fraction was not examined further by the registrant. Since this fraction contains ≥ 10 percent of the total recovered ^{14}C residues, further attempts must be made to release (via a combination of hydrolytic techniques [i.e, nondestructive acidic, basic, and/or enzymatic]) and identify ^{14}C-conjugates and/or determine the nature of ^{14}C incorporated into natural plant constituents of this fraction.

DEB reiterates its previous recommendation that in future ^{14}C plant metabolism studies, the nature of the unextracted residues (comprising ≥ 10 percent of total recovered ^{14}C activity) should be adequately characterized following appropriate hydrolytic and fractionation procedures.

5. The registrant, in his submitted ^{14}C lettuce/spinach metabolism study, has not accomplished what his own March 6, 1987 revised protocol had proposed to achieve (i.e., "Direct metabolites of ^{14}C-1,3-dichloropropenes present at or above 0.1 ppm will be isolated and characterized or identified").

   The Spinach Soluble II fraction, HPLC analysis yielded a discrete peak of intermediate polarity representing 8.5 percent or 0.10 ppm of total recovered ^{14}C residues and the HPLC analysis of the acid hydrolysate of this same fraction yielded a peak of intermediate polarity representing 10.8 percent or 0.21 ppm of total ^{14}C activity, neither of which was characterized or identified by the registrant.

   In addition, HPLC analysis of the Lettuce II C_{18} Sep-Pak H_2O eluate fraction yielded a single peak of intermediate polarity containing 2.8 percent and 0.04 ppm of total recovered ^{14}C activity. HPLC analysis of the C_{18} Sep-Pak methanol eluate fraction yielded four peaks of intermediate polarity, each represent- ing 1.5 to 2.8 percent or 0.02 to 0.04 ppm of total recovered ^{14}C activity.

   DEB recommends that in future ^{14}C plant metabolism studies, direct metabolites and tertiary degradation products of 1,3-D at or above 0.1 ppm must be isolated and characterized or identified.

B. A Metabolism Study of Soybeans Grown in Soil Treated with ^{14}C-1,3-Dichloropropenes (GH-C2032)

For the reasons given below, DEB concludes that the submitted soybean ^{14}C plant metabolism study does not satisfy the requirements of Section 171-4 (i.e., qualitative nature of the total terminal residue in soybeans has not been adequately identified/ characterized) and therefore, the study must be repeated. DEB's comments/conclusions are enumerated below.

1. In the submitted metabolism study, total ^{14}C residues, all at significant levels in green forage, soybean pods and vines, and soybeans (2.84, 5.37 and 5.18 ppm, respectively) were not adequately characterized/identified by the registrant; therefore, his hypothesis that (a) the metabolic products were not translocated to the bean and thus all of the bean residue is due to natural incorporation, and (b) that soybean field trash (pods and vines) samples consist primarily of incorporated ^{14}C activity or that there is no change in the character of the residue from the green forage to the fully mature trash stage are not adequately supported based on DEB's review of the submitted data.

2. DEB's comments above, under item A.2. for the lettuce/spinach metabolism study, are also applicable to the soybean metabolism study.

3. DEB's comments above under item A.3. for the lettuce/spinach metabolism study are also applicable to the soybean metabolism study. However, a few points specific to this study are worth noting. The registrant's observation in the current metabolism study that the ^{14}C residue in soybean green forage extracts and fractions (which were neither further fractionated nor characterized) appeared to be unchanged after 23 weeks in the freezer, does not satisfy DEB's earlier requirements for a storage stability study for the parent compound and its 3-chloro-2-propen-1-ol metabolite. In addition, the 50 percent methanol extract fractions of soybean pods and vines (trash) and soybean green forage #3, both of which, according to the registrant, would contain 1,3-D and its anticipated metabolites, if present, were derived from ^{14}C soybean samples stored frozen for 120 and 174 days, respectively, prior to HPLC analysis.

Neither 1,3-D, CAA or cis/trans CA were detected by cochromatography with the 50 percent methanol extract fractions by the registrant.

DEB reiterates its previous recommendation that in future ^{14}C plant metabolism studies all ^{14}C samples should be analyzed within 2 weeks of harvest and accompanied by a storage stability study for parent compound plus all anticipated metabolites if samples are not analyzed within this time interval.

4. DEB's comments above under item A.4. for the lettuce/spinach metabolism study are also applicable to the soybean metabolism study. However, a few points specific to this study are worth noting.

   In the submitted soybean metabolism study, DEB calculated that the "unextractable residue fraction" remaining following acid (1.2N HCl @ 100 °C for 4 hrs) hydrolysis of the methanol insoluble fractions of the soybean green forage #3, soybean trash (pods and vines) and soybean mature bean samples, contained 18.7 percent or 0.53 ppm; 19.6 percent or 1.05 ppm, and 10.0 percent or 0.51 ppm, respectively, of the total ^{14}C residue recovered from each original sample. None of these fractions were examined further by the registrant. Since each fraction contains $\geq$ 10 percent of the total recovered ^{14}C residues, further attempts must be made to release (via a combination of hydrolytic techniques [i.e., nondestructive acidic, basic, and/or enzymatic]) and identify ^{14}C-conjugates and/or determine the nature of ^{14}C incorporated into the natural plant constituents of each fraction.

   DEB reiterates its previous recommendation that in future ^{14}C plant metabolism studies the nature of unextracted residues (comprising $\geq$ 10 percent of total recovered ^{14}C activity) should be adequately characterized following appropriate hydrolytic and fractionation procedures.

5. As was the case with the lettuce/spinach metabolism study in A.5. above, direct metabolites of ^{14}C-1,3-D present in soybean extracts/fractions at or above 0.1 ppm were not isolated and characterized or identified.

Although the 50 percent methanol extracts of the mature soybean yielded only 16.3 percent or 0.84 ppm of the total recovered ^{14}C residues, further isolation and characterization or identification

of ^{14}C residues in this fraction will be required. For example, a subfraction of the 50 percent methanol extract, the Soluble II fraction yielded a discrete HPLC peak of intermediate polarity representing 2.2 percent or 0.11 ppm of total recovered ^{14}C residues, which was neither further isolated and characterized nor identified by the registrant.

Additionally, HPLC analyses of the Soybean Green Forage #3 and Soybean Trash (Pods and Vines) C_{18} Sep-Pak methanol eluate fractions yielded two and three major peaks, respectively, of intermediate polarity representing 8.8 percent (0.25 ppm), 8.4 percent (0.24 ppm), 7.2 percent (0.39 ppm), 5.3 percent (0.28 ppm), and 2.3 percent (0.12 ppm) of total recovered ^{14}C activity. Based on relative HPLC elution times, the first two peaks in each elution series appeared to be the same compound. However, none of the ^{14}C HPLC peaks observed in these fractions, which were postulated by the registrant to be metabolites or tertiary degradation products of 1,3-D, was further isolated and characterized or identified. The ^{14}C HPLC peaks did not cochromatograph with ^{14}C standards of 1,3-D, CAA, or cis/trans CA.

DEB recommends that in future ^{14}C plant metabolism studies, direct metabolites and tertiary degradation products of 1,3-D at or above 0.1 ppm must be isolated and characterized or identified.

Petitioner's Response to Deficiency Nos. A. 1, 2, 3, 4, and 5 and B. 1, 2, 3, 4, and 5

1. ^{14}C-1,3-D (DCP) Plant Metabolism Studies; Summary of December 9, 1988 meeting with EPA by Paul Lewer dated December 15, 1988.

"In summary, Dr. Kovacs recommended submitting new protocols to:

"a. Justify Dow not repeating the whole study, as he had requested in his review and,

"b. Explain as precisely as possible what we would propose to do instead (i.e., further extractions, fractionations, chromatography, etc.)

"Dr. Kovacs reported that new protocols are generally given a high priority by the Agency since they can be dealt with relatively quickly.

"Our meeting was very useful in clarifying several points from Dr. Kovacs review, as well as establishing scientific contact within the Agency. Overall, it confirmed that we should be able to complete this study to the satisfaction of the Agency without the need to repeat the field application of ^{14}C-DCP."

2. 1,3-D (DCP) Metabolism Study by Paul Lewer dated June 29, 1989.

"Reading the recent EPA review of our proposed protocol amendments for the above study suggests that some confusion appears to have arisen concerning the Agency's and our own, expectations. The following summary, in conjunction with that of 12/15/88 (copy attached), shows the position we had reached, at least as seen by us, following our December 9, 1988 visit with EPA.

"a. The original reports showed some ^{14}C-components in the 0.1-0.2 ppm range during high performance liquid chromatography (HPLC), which were proposed to be due to the overlapping of closely-eluting metabolites. In addition, all of the aqueous-organic extracts examined contained a relatively large amount of radioactivity which had no retention on a C_{18}-HPLC column, and were therefore either very polar or of high molecular weight. During our discussions with the reviewer, Dr. Martin Kovacs, we agreed to further fractionate these aqueous-organic-soluble ^{14}C-components to look for any major (i.e., > 0.1 ppm or > 10 percent of total ^{14}C) metabolites. The techniques to be used to do this were to be detailed in protocol amendments to be submitted to the Agency. This was done, and received Agency approval in Dr. Kovac's protocol amendment review of June 13, 1989.

"b. A major ^{14}C-fraction in most of the samples taken during the study was insoluble in aqueous-organic solvent mixtures ('insoluble fraction'). Our discussion with Dr. Kovacs led us to conclude that the Agency's main concern was with the possible presence of conjugates of 'moieties of concern' which might be insoluble in the solvents tried. We agreed to re-analysis these fractions more thoroughly and planned to do this by the methods outlined for the very polar aqueous-organic-soluble material discussed in a. above (the original report showed that the 'insoluble fraction' could be largely solubilized by use or either base of acid and that the solubilized material had no retention on C_{18}-HPLC). Dr. Kovacs referred us to a previous DCP metabolism study (in sugar beets) and the techniques used in that case. While the techniques used in that study (e.g, ion-exchange) were appropriate and in keeping with the technology available at that time (1973), we feel that a combination of those methods with the most modern high resolution chromatographic techniques (i.e., HPLC) is more appropriate. The question of methods used to characterize the 'insoluble fraction' was not explicitly addressed in the amended protocols since the methods to be used were a combination of those described elsewhere in more detail.

"c. At the 12/9/88 meeting, we understood that it was agreed that, provided we could demonstrate the integrity of the samples since their 1987 collection, the same samples would be valid for further analyses. In this respect we took it that re-analysis would be specifically addressing the points discussed in a. and b. above - i.e., the probability of closely-eluting metabolites in the intermediate-polarity solution range and the very polar materials, however solubilized, - <u>not</u> to try to detect and quantify the parent DCP and/or the chlorallyl alcohols, which had already been shown to be undetectable in the tissues collected in 1987. In order to address the Agency's concern regarding the storage stability of DCP and the chlorallyl alcohols in the plant matrices, and in typical aqueous-organic extracts from these matrices, and thus to demonstrate that the absence of those compounds in the tissues examined was not due to chemical decomposition, it was agreed that a storage stability study

would be performed using traditional residue methodology. Therefore, this point was not explicitly discussed in our amended protocols, since our understanding was that the storage stability experiments would be done. Subsequently, the analyses performed since the December 9, 1988 meeting showed that the ^{14}C-profiles in each tissue sample were the same as those in the original report, as far as the ^{14}C components that were originally detected were concerned (i.e., regardless of any question of the storage stability of DCP/CAA).

"d. Further Action.

A preliminary storage stability study of ^{14}C-DCP and CAA in lettuce homogenate is planned, to start June 27, 1989. This will investigate the stability of these compounds when mixed with a lettuce homogenate at approx. 0.5 ppm, a value in the middle of the range of those found during the early part of the study. Samples from this experiment will also be used to check the chemical stability of both compounds in aqueous-organic solvent extracts from the homogenates. Storage conditions will reproduce those used for the storage of the tissues from the treatment plot in 1987. Aliquots from the samples will be taken for analysis approx. every seven days over a period of approx. thirty-five days. The total time required for collection and analysis of the data would be expected to be in the region of forty-five days. The data from this experiment are not intended to substitute for a full storage stability study, but to provide supportive evidence in the interim period until the completion of such a full study.

We, therefore, feel that we can demonstrate that had DCP or the chloroallyl alcohols been present in the plant samples originally analyzed, they would have been detected. With completion of the short-term storage stability experiment the data should continue to support this conclusion. Additionally, we will continue to examine methods of characterization and fractionation of the 'insoluble' components, to look for possible residues of concern and/or characterize these compounds as natural plant constituents which have incorporated. ^{14}C. We will be prepared to meet with Dr. Kovacs again upon completion of the storage stability test to resolve these

differences and outline a clear path to complete these studies. We feel very strongly that the data presented to date are valid and will continue to support this position."

3. 1,3-D Plant Metabolism Studies by Paul Lewer dated August 24, 1989.

"Dr. Martin Kovacs, of the EPA, recently reviewed two reports: 'A metabolism study of lettuce and spinach grown in soil treated with ^{14}C-1,3-dichloropropene' (GH-C 2031) and 'A metabolism study of soybeans grown in soil treated with ^{14}C-1,3-dichloroprone' (GH-C 2032). In our meeting with Dr. Kovacs (12/9/88) and in subsequent correspondence, he expressed concern that the non-detection of 1,3-dichloropropene (DCP) and 3-chloroallyl alcohol (CAA) during these studies might be due to complete loss or decomposition of these materials during storage of the harvested tissues. This letter summarizes my recent experimental work to address these concerns, and demonstrates that if DCP or CAA had been present in the tissues when harvested, they would have been stable under the storage conditions used, and therefore would have been detected and characterized.

"The storage stability properties of [^{14}C]-1,3-dichloropropene (DCP) and [^{14}C]-chloroallyl alcohol (CAA) were investigated in separate experiments, as a function of time, using lettuce and methanol (MeOH) extracts of lettuce. This tissue was chosen since it was one of those used in the original study, was readily available and, in view of its high water content, was the matrix most likely to show any artifactual hydrolysis of DCP to CAA, or loss of these compounds through volatilization . . . .

"In summary, the data demonstrated that:

"a. DCP, and CAA-fortified lettuce homogenates sustained losses of the compounds over a 44 day time-course consistent with their volatilities and water-solubilities. After 28 and 44 days frozen storage, DCP-fortified lettuce contained 70% and 48% of the zero-time concentration of DCP; CAA-fortified lettuce contained 94% and 83% of the zero time concentration of CAA, respectively. In view of the fact that, in the original study (GH-C 2031), all analyses for these two compounds (by steam distillation/

HPLC) were performed within 10 days of tissue harvesting, the present data clearly showed that, had the tissues contained either DCP or CAA, they would have been stable, detectable (if above the limits of detection) and characterizable.

"b. DCP and CAA were completely stable in MeOH extracts of lettuce when stored under the same conditions as were the extracts in the study reported in GH-C 2031. Therefore, any MeOH extracts made and stored under these conditions would be valid for DCP/CAA analysis for at least 44 days.

"c. No measurable amounts of any putative metabolites were formed under the storage conditions used, nor were any of the metabolic products reported in the previous study (GH-C 2031) detected.

We would welcome further discussion with Dr. Kovacs on the matter of the storage stabilities of DCP and CAA. Further, we would like to discuss our plans for analysis of the residues from the 1987 tissues, especially those insoluble in aqueous/organic solvent mixtures. Finally, in the meanwhile, we will continue to develop analytical methods for these 'insoluble' residues."

DEB Comments/Conclusions Re: Deficiency Nos. A. 1, 2, 3, 4, and 5 and B. 1, 2, 3, 4, and 5

It appears the petitioner misunderstood Dr. Kovacs at the December 9, 1988 meeting. However, any misunderstanding the petitioner had from the December 9, 1988 meeting should have been resolved by DEB's June 8, 1989 review of M. Kovacs which clearly indicated that new plant metabolism studies were required.

The storage stability data in this submission on 1,3-Dichloropropene and chloroallyl alcohol does not revise DEB's position that new plant metabolism studies are required with all ^{14}C samples frozen and analyzed within 2 weeks of harvest or accompanied by a storage stability study for the parent compound plus all anticipated metabolites if samples are not analyzed within this time interval. The nature of the total terminal residue has not been adequately characterized and likely includes other metabolites (e.g., malonic acid, 3-hydroxypropionic acid, pyruvate, propionic

acid, etc.) for which storage stability data are unavailable. The total terminal residue includes all components of the resultant residue whether or not they are toxic enough to be of concern. Additionally, as indicated in Attachment 1 - Position Document on Effects of Storage on Validity of Pesticide Residue Data, storage stability studies should normally be run concurrently with the storage of treated samples.

Furthermore, the original plant metabolism studies were not invalidated because of storage stability concerns, alone. Neither the soluble or insoluble extracts/fractions were adequately characterized and accordingly any further attempt to validate the original plant metabolism studies, by reanalyzing samples stored since 1987 for which the total terminal residue was not originally characterized, is futile. Additionally, new concerns have arisen concerning the use of a 70% sand-sandy loam soil in both the metabolism studies.

In conjunction with the requirement for the new plant metabolism studies, the petitioner was sent a copy of the Pesticide Assessment Guidelines Subdivision O, Nature of the Residue: Plants, in DEB's June 8, 1989 review of M. Kovacs for guidance in completing the new plant metabolism studies. Further in DEB's July 7, 1988 review of M. Kovacs, the petitioner was specifically directed to use exhaustive Soxhlet extraction procedures, different strengths of acid and/or base, and enzymatic techniques in order to release conjugated/bound residues in the insoluble fractions. DEB notes that the petitioner has utilized basic and enzymatic extraction procedures recently under PP#9G3771 for Dowco 429 on corn.

The petitioner needs to make a more diligent effort in the new metabolism studies to determine the nature of the total terminal residue with a proposed metabolic pathway so that a determination of the chemical definition of the total toxic residue can be made. It is more important to establish what is there (i.e., the identity or character of the total terminal residue) than systematically deduce what is not in the terminal residue (i.e., the parent and its primary metabolites) as was accomplished for the original ^{14}C lettuce/spinach and soybean metabolism studies.

Other Considerations

1. A report in EPA files titled "Factors Influencing Diffussion and Nematode Control by Soil Fumigants" dated November 29, 1957 by the Dow Chemical Company indicates certain factors such, as soil temperature, depth of soil injection, soil moisture and soil

type, significantly impact the diffusion of soil fumigants such as 1,3-D.

In particular, molecular soil diffusion is less restricted in sandy soils than in clay soils with smaller size particles and pores; and that soil organic matter interacts with the fumigants; both factors which can reduce the fumigant's effectiveness, require higher application rates and may affect the persistence of the fumigant and its metabolites. Consequently, current labeling for 1,3-D recommends application rates for vegetable and field crops in muck or peat soils generally twice the application rate required in mineral soils and higher application rates in mineral soils with finer textured heavier soils such as clay loam.

DEB notes that both the soybean and lettuce/spinach plant metabolism studies previously submitted were conducted in a sandy loam soil of 16 percent silt, 70 percent sand, 14 percent clay, 1.98 percent C, 12.9 percent 1/3 bar moisture and a pH of 5.5. Because soil type may impact on the persistence of 1,3-D in the soil and resulting plant residues, the new plant metabolism studies must be conducted in different soils (i.e., sandy loam and clay loam).

2. The previously submitted plant metabolism studies contained no individual sample storage records, actual chromatograms or radiochemical validation of the proposed analytical procedures. The required new plant metabolism studies should include individual sample storage records and summaries with a complete sample storage history including time and conditions of frozen storage as well as time and conditions before frozen storage, extraction, cleanup and analysis; copies of representative chromatograms; and radiochemical validation of the proposed analytical procedures.

Attachments: 1

Attachment No. 1: Position Document - Effects of Storage (Storage Stability) on Validity of Pesticide Residue Data.

CC without Attachment: PMSD/ISB, RF, Circu, 1,3-Dichloropropene Registration Standard File, Reviewer - Otakie

H7509C:DEB:G.Otakie:CM#2:Room 804:557-7484:mb:12/20/89
RDI:D.Edwards:12/20/89:R.Loranger:12/20/89

53492:I:Otakie:C.Disk:KENCO:12/04/89:ka:sw:vo:ka:vo:ek:ka