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

JUL 7 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 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]

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Introduction

Residue Chemistry Branch (RCB) has been requested to review and evaluate two plant metabolism studies: 1) Lettuce and Spinach Grown in Soil Treated With ^{14}C-1,3-Dichloropropenes (GH-C2031), and 2) Soybeans Grown in Soil Treated With ^{14}C-1,3-Dichloropropenes (GHC-2032), both submitted by Dow Chemical U.S.A., Midland, MI for the soil fumigant TELONE® (EPA Registration No. 464-511). They were submitted in order to determine their acceptability in fulfilling the data gap identified in Section 171-4 of the Residue Chemistry Chapter in the Telone Registration Standard issued on August 12, 1985.

In his March 25, 1988 letter to H. Toma (RD/EPA), Robert W. Morgan (Manager, Regulatory Planning Agricultural Products Department, Dow Chemical Company) requested that the requirement for field residue studies of 1,3-Dichloropropene (1,3-D) be waived and that the preplant use of this chemical as defined by registered labeling for products in which it is contained as an active ingredient be declared to be a nonfood use as defined under Section 408 of FFDCA.

Summary of Deficiencies Remaining to be Resolved

- 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-D. Therefore, this use in definitely a food use requiring a full set of residue data requirements.

- The nature of the total terminal residue in lettuce, spinach, and soybeans has not been adequately characterized (see RCB Comments/Conclusions A(5)and B(5) that follow in this review).

- 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 RCB's review of the submitted data. To validate this hypothesis, the registrant may want to utilize ^{37}Cl-labeled 1,3-D in some additional metabolism studies and utilize all available techniques to release bound/conjugated residues in his samples (see RCB's conclusions given later in this review).

- RCB 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).

- The registrant has not submitted storage stability data that will support the submitted ^{14}C residue data.

- RCB 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.

Background

In 1985, in conjunction with the Telone Registration

Standard, the RCB reviewed all available data regarding the metabolism of Telone (1,3-D) in plants. At that time, it was determined that the nature of the residue in plants was not adequately understood. Therefore, the following additional data were required:

- Data depicting the distribution and metabolism of [^{14}C]1,3-D in soybeans and lettuce harvested at regular intervals which encompass seedling and mature growth stages following a single preplant soil injection application at 6 to 8 inches below the soil surface with the 92 percent RTU formulation at a rate sufficiently high to permit complete characterization of ^{14}C-residues. If a significant proportion of the terminal residues are unextractable by the solvent(s) used, then analyses must include hydrolysis and reextraction of plant residues to determine the nature of bound or conjugated residues.

In response to this data requirement, Dow Chemical U.S.A. submitted proposed protocols for completion of the lettuce and soybean studies and requested approval or suggestions for appropriate modifications.

The RCB review, (D. Edwards, November 5, 1986), suggested the following additions and modifications to the protocols in order to bring them into conformity with specific requirements of the Registration Standard:

1. In addition to the method of analysis (radio-HPLC) in the proposed protocol, samples should be analyzed for all identified metabolites of potential toxicological concern using methods (preferably GC) which may be used for enforcement of tolerances to be established in the future. In this way, the efficiency of extraction procedures used in the potential regulatory methods can be validated.

2. The proposed protocol states that samples will be "held in a freezer until analyzed" but does not state the approximate length of time that samples will be stored. Since the stability of 1,3-D in/on frozen macerated samples of lettuce and potatoes was poor (after 75 days, 55 to 66 percent and 27 to 40 percent of initial level remained in/on lettuce and potatoes, respectively--refer to the Storage Stability section of the Residue Chemistry Chapter of the Telone Registration Standard), we recommend that 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.

3. The proposed protocol gives no indication as to what procedures will be used to identify the nature of residues not extracted with the polar and nonpolar solvents, should they occur. 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 the aforementioned protocol review, Dow submitted for informational purposes and RCB reviewed (J. Garbus, June 1, 1987) revised "final" protocols for lettuce and soybean plant metabolism studies both dated March 6, 1987 which incorporated RCB's earlier comments and suggestions into these "final" protocols as follows:

RCB suggested (D. Edwards, November 5, 1986):

1. In addition to the method of analysis (radio-HPLC) samples should be analyzed for metabolites using methods which could be used for enforcement (i.e., GC).

   Dow has added GC to the description of analytical methods. The pertinent sentence in the protocol now reads ". . . the extracts will be examined by radio-HPLC and GC for the presence of 1,3-dichloropropene and metabolites."

2. As the stability of 1,3-D in frozen samples is poor, we recommend that frozen samples be analyzed within 2 weeks of harvest and that frozen samples be fortified with Telone and analyzed to determine the degree of loss during storage.

   Dow has added a paragraph to the final protocol to the effect that a separate storage stability study will be undertaken.

3. Attempts must be made to release and identify ^{14}C-conjugates and/or determine the nature of ^{14}C incorporated into natural plant constituents.

> Dow's protocol now includes the statement, "Insoluble residues will be examined for conjugate formation and for natural incorporation of ^{14}C-activity."

RCB concluded in its J. Garbus, June 1, 1987 protocol review:

> Dow has revised its protocols for plant metabolism studies of 1,3-dichloropropene to include suggested additions and modifications presented in RCB's review of the draft, proposed protocol.

Recommendations

1. RCB recommends, at this time, that the registrant's request for a waiver of field residue studies for 1,3-D not be granted since the proposed use is definitely a food use.

2. Based on RCB's comments/conclusions, which follow in this review under the Present Considerations section, RCB concludes that the registrant's submitted (lettuce, spinach) and soybean plant metabolism studies do not satisfy the requirements of Section 171-4 (i.e., the qualitative nature of the total terminal residue in plants has not been adequately characterized and therefore these studies should be repeated.

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. For guidance in the conduct of the resubmitted metabolism studies, the registrant is directed to the Pesticide Assessment Guidelines Subdivision O Addendum on Data Reporting, Nature of the Residue: Plants, which is attached to this review.

4. For the reasons given in Recommendation 1 above, RCB also recommends that the preplant use of this chemical not be declared as requested by the petitioner, to be a nonfood use as defined under Section 408 of the FFCDA.

RCB's Comments/Conclusions Resulting From the Review of This Submission

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 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. RCB'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. RCB 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 <u>and</u> <u>GC</u> for the presence of 1,3-D and metabolites.

   In the submitted lettuce/spinach metabolism study, RCB notes that <u>none</u> 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. RCB 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. RCB had previously recommended in its D.F. Edwards November 5, 1986 plant metabolism protocol review that ^{14}C samples be analyzed <u>within</u> 2 weeks of harvest, and further that frozen control samples fortified with Telone and anticipated metabolites

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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-dichloropropene 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.

RCB 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 RCB's earlier requirements for a storage stability study for the parent compound and its 3-chloro-2-propen-1-ol metabolite.

RCB 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. RCB had previously suggested in its D.F. Edwards November 5, 1986 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, RCB 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 fraction was not examined further by the registrant. Since this 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 natural plant constituents of this fraction.

RCB reiterates its previous recommendation that in future ^{14}C plant metabolism studies, the nature of the unextracted residues (comprising $\geq$ 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 representing 1.5 to 2.8 percent or 0.02 to 0.04 ppm of total recovered ^{14}C activity.

RCB 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, RCB 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 characterized/identified) and therefore, the study must be repeated. RCB'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 RCB's review of the submitted data.

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

3. RCB'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 RCB'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 co-chromatography with the 50 percent methanol extract fractions by the registrant.

RCB 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 parent compound plus all anticipated metabolites if samples are not analyzed within this time interval.

4. RCB'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, RCB 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.

RCB reiterates its previous recommendation that in future ^{14}C plant metabolism studies the nature of unextracted residues (comprising $\geq$ 10% 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.

RCB 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.

Other Considerations

Prior to the 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. These protocols should also address RCB's additional concerns arising from the review of the current metabolism studies. These are: 1) the identity of radiolabeled impurities arising from the test material; 2) inclusion of 1,2 dichloropropane (a major [0.5%] impurity component of Telone II formulations) as a test substance in the resubmitted ^{14}C metabolism studies; 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 on Data Reporting, Nature of the Residue: Plants for general guidance in this area, a copy of which is attached to this review.

Present Considerations

Dow Chemical U.S.A. has submitted two reports of plant metabolism studies for plants grown in 1,3-D-treated soil, as required under provisions of the subject Registration Standard and the Data Call-In Notice for Residue Chemistry Data for 1,3-Dichloropropene dated May 14, 1986 as follows:

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

Application

The radiolabeled material used in this study was UL-^{14}C-D (cis-trans mixture) with a specific activity of 1.31 Ci/mole diluted with unlabeled compound (Telone II) to a final specific activity of 0.898 Ci/mole (17,900 DPM per ug) with radiochemical purity of 96.9 percent and cis-trans isomer ratio approximately equal.

The test site was Field No. 2 of the Dow Agricultural Field Station, Midland, MI, with the soil classified as a Londo sandy loam. The test plot was 1 ft by 6 ft. The ^{14}C-1,3-D was drawn up into a 50 mL Eppendorf Repeator Pipette and injected 8 inches deep into the soil in 1.0 mL amounts along the two long (6 ft) edges of the plot. Both treatment rows received 19 injections at a spacing of 3.8 inches. After application, the plot was lightly tamped. The application of 38 mL of neat ^{14}C-1,3-D to the 6-ft^2 plot was equivalent to applying 36 GPA of Telone II with a total radioactivity applied of 372 mCi.

Immediately after application, lettuce and spinach were planted in two rows, each 1-inch east of the injection rows. On June 19, 25 days after application, lettuce was planted in a single row down the center of the plot.

Sample Handling and Storage Stability

In summary, the registrant states:

> The spinach was harvested July 6, 42 days after planting, and the root systems removed to prevent regrowth. The lettuce of the original planting was harvested July 21, 57 days after planting, and these roots removed also. The lettuce of the second planting was harvested three times, 39, 52, and 75 days after planting (July 28, August 10, and September 2, respectively), and good regrowth was obtained.

Then, according to the registrant, the following techniques were carried out:

> The plants were excised about one-half inch above ground level, put into plastic bags, and placed in an insulated box containing dry ice. The samples were brought into the laboratory, weighed, and when completely frozen (within 2 hr), homogenized by blending with dry ice in a Waring Blender. The blends were put into glass bottles, lightly capped, and held in a freezer overnight to allow the CO_2 to escape. Samples for analyses were weighed out the next day in the frozen state. The bulk sample was never allowed to thaw.
>
> Repeat analyses performed several weeks after the initial analyses indicated that the nature of the residue did not change during storage in the freezer.
>
> Samples of lettuce from plants grown in untreated plots were obtained for control samples, and prepared for analyses and stored, as above.
>
> Soil core samples were taken 25 days and 106 days (September 8) after treatment. They were hand-blended without drying. Subsamples were analyzed and the remainder of the samples were frozen within 24 hours.

Sample Analysis

The registrant's work indicated that:

> The homogenized plant and soil samples were analyzed for total residual ^{14}C-activity by combustion, for volatile ^{14}C residues by steam distillation, and for ^{14}C metabolic products by solvent extraction and HPLC analysis.

Characterization of the Residues

The registrant attempted to characterize their residues by HPLC elution times relative to the reference compounds, by volatility or solvent distribution characteristics by stability to acid hydrolysis, and by dry weight content of ^{14}C activity. The ether fraction of the 106-day soil steam distillate was examined by GC-MS.

Results and Discussion

Total ^{14}C Residues

Total ^{14}C-activities reported as DPM/G and ppm as 1,3-D equivalents on a field weight and dry basis following combustion of the five plant samples were reported by the registrant as follows:

Table 1

Sample	Weight g	Days After: Appl.	Days After: Plant	Total ^{14}C-Activity DPM/G	ppm[a]	ppm[b]
Spinach[c]	2485	42	42	34383	1.92	28.5
Lettuce I[c]	153	57	57	32264	1.80	18.8
Lettuce II[d]	1042	64	39	23555	1.32	17.6
Lettuce III[e]	1314	77	52	9072	0.51	7.9
Lettuce IV[e]	846	100	75	5988	0.34	6.2

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[a] ppm as 1,3-D equivalent, field weight basis.
[b] ppm as 1,3-D equivelant, dry basis.
[c] Planted at the time of application.
[d] Planted 25 days after application.
[e] Regrowth of Lettuce II.

The registrant comments that the total ^{14}C residue in plants are probably high because the maximum recommended application rate for vegetables in mineral soil is 15 GPA, not 36 GPA as used in the study.

The total ^{14}C-activity found in the soil samples taken at time of second planting (25 days after application) and after removing the final lettuce crop was reported by the registrant as follows:

Table 2

Days After Application	Depth (in)	Weight (g)	Total ^{14}C Activity DPM/g	ppm	Percent
25[a]	0-6	1009	113795	6.4	38.4
	6-12	1288	143990	8.0	48.2
	12-18	1296	50719	2.8	13.4
106[b]	0-6	726	91092	5.1	44.1
	6-12	742	87747	4.9	43.4
	12-18	902	16631	0.9	10.0
	18-24	781	4639	0.3	2.4

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[a] Four 1.5-inch diameter probes.
[b] Six 1-inch diameter probes.

The registrant calculates that assuming that most of the residual ^{14}C activity at final harvest was present in a volume of 2-ft wide by 7-ft long by 18-in deep, only about 8 percent of the applied ^{14}C activity was found 106 days after application.

Characterization of the Plant Residues

Steam distillation analyses of all plant samples which indicated that only 2 percent of loss of the ^{14}C residue of the lettuce and spinach was volatile enough to distill lead the registrant to conclude that of the total ^{14}C present, less than 3 percent consisted of 1,3-D and/or 3-chloro-2-propene-1-ol (CAA). Over 80 percent of ^{14}C-1,3-D or ^{14}C-CAA added to control lettuce samples was recovered in the steam distillate.

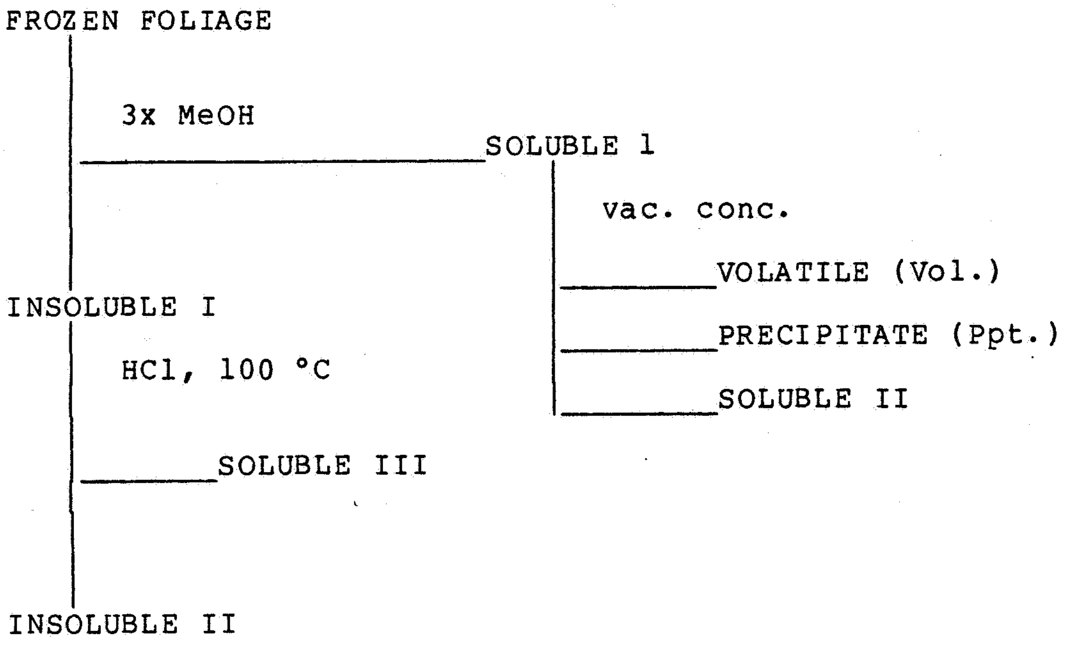
Frozen plant samples were extracted with three solvents, diethyl ether, methanol and 50 percent methanol. Diethyl ether extracted only 8 percent of the total ^{14}C residue; therefore, these extracts were not examined further.

Methanol extracted about 60 percent of the spinach sample ^{14}C activity and 40 percent of the lettuce residue. Those extractions and subsequent separations are summarized by the registrant below.

Table 3
(Figure 5 of Petitioner's Text)

Separations Performed on Plant Samples and Control Samples Spiked with Reference ^{14}C-Labeled Compounds

OUTLINE



(Cont'd next page)

Table 3

Separations Performed on Plant Samples and Control Samples Spiked with Reference ^{14}C-Labeled Compound (Cont'd)

DATA SUMMARY

	Percent of Initial ^{14}C Activity						
	Insoluble		Soluble				
	I	II	I	II	III	Vol.	Ppt.
Spinach (358959 DPM)	[d]	[d]	65.9	64.1	[d]	0.5	6.9
Spinach[a] (1782071 DPM)	30.6	8.6	63.3	61.6	18.9	0.4	2.6
Lettuce I (813698 DPM)	59.2	[d]	40.9	34.0	[d]	1.0	6.3
Lettuce II (589582 DPM)	58.2	[d]	38.9	32.4	[d]	0.6	6.3
Lettuce II[b] (1222033 DPM)	57.2	19.6	39.0	27.1	33.3	0.6	7.4
Lettuce II[c] (588168 DPM)	58.0	[d]	39.5	32.5	[d]	0.7	6.2
Lettuce III (228977 DPM)	62.2	[d]	39.0	32.7	[d]	0.7	5.7
Lettuce IV (155269 DPM)	68.6	[d]	41.8	31.4	[d]	0.7	6.0
Control + ^{14}C-1,3-D (1034596 DPM)	0.2	[d]	116.4	0.4	[d]	103.4	0.0
Control + ^{14}C-CAA (3498140 DPM)	0.1	[d]	94.7	80.4	[d]	19.2	0.5

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[a] 7 days in the freezer.
[b] 47 days in the freezer.
[c] 160 days in the freezer.
[d] Not determined.

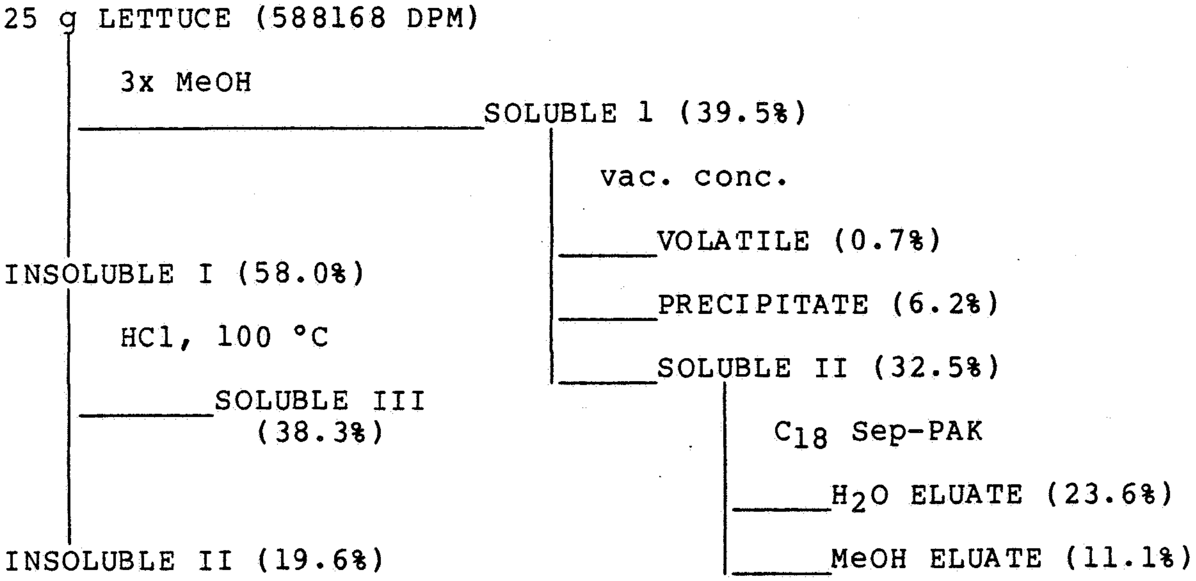
HPLC analyses performed on the Soluble II fractions derived from the spinach and each of the four lettuce samples indicated the presence of very polar material eluting at the apparent void volume; several unidentified components of intermediate polarity, and no CAA (1,3-D would not be in this fraction). The registrant noted with concern the nature of the very polar fraction which constituted about one-half of the fraction examined; however, the chromatograms indicated that there was no qualitative change in the lettuce residues over time.

Acid hydrolysis (1.2N HCl, 100 °C, 4 hr) of the spinach and lettuce II, Soluble II fractions, as a test for conjugation, had little effect on the very polar constituents as evidenced by submitted HPLC histograms which indicated a similar ^{14}C elution pattern to the original spinach and lettuce II HPLC histograms. Acid hydrolysis (1.2N HCl, 100 °C, 4 hr) of the lettuce II, Insoluble I fraction (58.0% total ^{14}C activity), solubilized an appreciable amount of the residue (Soluble III fraction 38.3% total ^{14}C activity). Acid hydrolysis of the spinach Insoluble I fraction (30.6% total ^{14}C activity) also solubilized an appreciable amount of this residue (Soluble III fraction 18.9% total ^{14}C activity). HPLC histograms of these solubilized fractions indicated the generation of mainly more very polar substances as evidenced by the lack of discrete peaks of intermediate polarity. The registrant concludes that no evidence was obtained for the presence of soluble or insoluble conjugates in these samples.

The registrant furthermore concludes that the data strongly suggest that the residues in these samples may be naturally incorporated ^{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. To support this conclusion, the registrant attempted to search for a fraction containing an exceptionally high concentration of ^{14}C activity. To do this, the dry-weight concentration of ^{14}C was measured, either by combustion of dry fractions, or by counting and drying separate aliquots of the soluble fractions.

An outline of this latter approach to isolate fractions containing high ^{14}C activity as applied to the lettuce II sample was provided by the registrant in Table 4 that follows. This approach involved separation of the Soluble II fraction into two separate fractions (H_2O eluates) and (MeOH eluate) based on the retention characteristics of the extract on a C_{18} Sep-Pak column. The ^{14}C concentration data expressed as ppm 1,3-D equivalent dry basis in Table 4 indicate that only one fraction, the methanol eluate of the Sep-Pak contained an elevated concentration of ^{14}C activity. The registrant states that the remaining fractions represent natural incorporation of ^{14}C residues because of their equal ^{14}C activity.

Table 4. Separations Performed on Lettuce II



^{14}C CONCENTRATION, as ppm 1,3-D Equivalent, Dry Basis

Lettuce	17.6
Insoluble I	15.8
Insoluble II	14.7
Soluble II	13.5
Soluble III	12.8
Precipitate	18.0
H_2O Eluate	9.3
MeOH Eluate	55.0

An HPLC histogram of the H_2O eluate fraction from the C_{18} Sep-Pak column indicated a peak of very high polarity which supported the registrant's contention that this fraction is composed of incorporated ^{14}C activity.

An HPLC histogram of the methanol eluate fraction from the C_{18} Sep-Pak column resulted in numerous peaks of intermediate polarity, the four largest of which did not correspond to the retention times of either CAA or the 3-chloroacrylic acids. According to the registrant, it is likely that this fraction would contain the metabolites of 1,3-D.

The registrant calculates, based on HPLC chromatograms of spinach and lettuce Soluble II fractions, that those peaks (or substances) of intermediate polarity account for 8.8 percent (1 peak for spinach) and 3.9 to 4.5 percent (several peaks for lettuce) of the total ^{14}C residue in each crop.

Characterization of the Soil Residues

Steam distillation of soil samples indicated that 15 and 6 percent of total ^{14}C residues were volatilized from 25- and 106-day soil studies, respectively. Concentration of the ether phase of a 25-day soil distillate, and examination by HPLC indicated a major unidentified volatile component slightly less polar than 1,3-D.

Extracting the soil samples with water-diethyl ether mixture (10 g soil, 15 mL water, 70 mL ether) released 14.9 to 15.7 and 6.0 to 11.3 percent, respectvevly of total ^{14}C activity in the 25- and 106-day soil samples. Concentration of the ether phase of a 106-day soil extract and analysis by HPLC produced a major peak with a retention time corresponding to 1,3-D. Concentration of the steam distillaton ether fraction of the 106-day soil and HPLC analyses also indicated the presence of apparent 1,3-D. However, examination of the latter concentrate by GC-MS failed to reveal a chlorine-containing compound and, therefore, confirm this conclusion. Reexamination of these isolates by isocratic HPLC indicated that the major ^{14}C-containing peak was not 1,3-D, but had a retention time longer than the cochromatographed 1,3-D standard. The registrant concludes that this isolated volatile, nonpolar soil metabolite may be a terminal and minor product of the degradation of 1,3-D by soil microorganisms.

Conclusions Drawn by the Registrant

Overall the registrant concluded that the submitted metabolism data for spinach and lettuce indicated that there are no residues of concern in these leafy vegetable crops grown in soil treated with ^{14}C-1,3-D. In addition, if significant amounts of 1,3-D, CAA, or 3-chloroacrylic acid had been part of the residue, they would have been found by the analytical procedures used. Conjugated or bound direct metabolites of 1,3-D do not appear to be present. According to the registrant, this study therefore supports the long-held assumption that the recommended application of 1,3-D as a soil fumigant can be classified as a nonfood use.

B. A Metabolism Study of Soybeans Grown in Soil Treated with ^{14}C-1,3-Dichloropropenes (GH-C203)

Application

Both the radiolabeled material used in this study and the test site selected were previously described in the spinach and lettuce metabolism study.

Immediately after application, the two treatment rows were planted with soybean seed at a spacing of 2 inches (planting #1). Twenty-five days after application, soybean seeds were planted in a single row down the center of the plot, and these plants were grown to maturity (planting #2). Thirty-five days after application, additional seeds were planted in the gaps of the center row which became planting #3.

Sample Handling and Storage Stability

The plants of plantings #1 and #3 were harvested on July 21 and August 3, 1987, respectively, for analysis at the green forage stage at which point the plants were just beginning to bloom. These plants were harvested and stored for analysis in the same manner previously described for spinach and lettuce.

Then, the following techniques were carried out:

> The plants of planting #2 were harvested on October 19, 1987, when fully mature. The plants were excised 1 in above ground level and air-dried overnight in the greenhouse. The beans were separated from the pods, the vines were sectioned, and the samples again air-dried overnight. The pods and vines were combined to form the field trash sample. The bean and trash samples were weighed, homogenized using a Wiley mill, and sampled for the analyses. These samples (in glass) were then

placed in a freezer, about 48 hr after removal from the plot.

Repeat analyses performed several weeks after the initial analyses indicated that the nature of the residue did not change during storage in the freezer.

Samples of green froage and mature soybean from plants grown in untreated plots were obtained for control samples, and prepared for analyses and stored as above.

Soil core samples were taken 25 days and 157 days after treatment. They were hand-blended without drying. Subsamples were analyzed and the remainder of the samples were frozen within 24 hours.

Sample Analysis

Sample analysis was conducted in the same manner as previously described for spinach and lettuce samples.

Characterization of the Residues

According to the registrant, the residues were characterized by their HPLC elution times relative to the reference compounds, by their volatility or solvent distribution characteristics, by the stability to acid hydrolysis, and by their dry weight content of ^{14}C-activity. Three fractions were examined by GC-MS.

Results and Discussion

Total ^{14}C Residues

Total ^{14}C-activities reported as DPM/G and ppm as 1,3-D equivalents on a field weight and dry basis following combustion of the four plant samples were reported in Table 1 (see the following page).

The registrant comments that "The total ^{14}C residues in plants must be considered abnormally high because 1) the recommended application rate for soybeans (row treatment, mineral soil) is 6 GPA, not the 36 GPA used in the study, and 2) all of the plants were stunted during the early growth period."

Table 1

Sample	Weight (g)	Days After: Appl.	Days After: Plant	Total ^{14}C Activity DPM/G	ppm[a]	ppm[b]
Green Forage (Planting #1)	118	57	57	138686	7.75	36.3
Green Forage (Planting #3)	204	70	35	50877	2.84	15.2
Mature Bean (Planting #2)	942	147	122	92787	5.18	5.59[c]
Field Trash (Planting #2)	734	147	122	96119	5.37	5.80[d]

---

[a] ppm as 1,3-D equivalent, field weight basis.
[b] ppm as 1,3-D equivalent, dry weight basis.
[c] Bean contained 55.3 percent of total activity at harvest.
[d] Trash contained 44.7 percent of total activity at harvest.

The total ^{14}C-activity found in the soil samples taken at time of planting #2 (25 days after application) and after removing the mature crop was reported by the registrant as follows:

Table 2

Days After Application	Depth (in)	Weight (g)	^{14}C Activity DPM/G	ppm	Percent
25[a]	0-6	1009	113795	6.4	38.4
	6-12	1288	143990	8.0	48.2
	12-18	1296	50719	2.8	13.4
157[b]	0-6	627	103550	5.8	38.6
	6-12	781	105055	5.9	48.8
	12-18	844	19620	1.1	9.9
	18-24	1079	4132	0.2	2.6

---

[a] Four 1.5 inch diameter probes.
[b] Six 1 inch diameter probes.

Characterization of the Green Forage Residues

Steam distillation analyses of all plant samples, which indicated that only 3 percent of the ^{14}C residues of the

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soybean green forage and trash were volatile enough to distill, lead the registrant to conclude:

> . . . that of the total ^{14}C present less than 5 percent consisted of 1,3-D and/or 3-chloro-2-propene-1-ol (CAA). About 80% of the ^{14}C-1,3-D or ^{14}C-CAA added to control forage, trash or soybean samples was recovered in the steam distillate.

According the registrant, the ether phases of the green forage distillates were concentrated and examined by GC-MS and HPLC. No 1,3-D or other recognizable chlorine-containing compound was found by GC-MS and the HPLC analyses revealed that the concentrates contained a single ^{14}C-containing compound with a t_R about 2 minutes longer than 1,3-D. The ^{14}C-containing compound was confirmed by cochromatography with 1,3-D, and thus the resulting data indicate that 1,3-D was not present in the green forage samples.

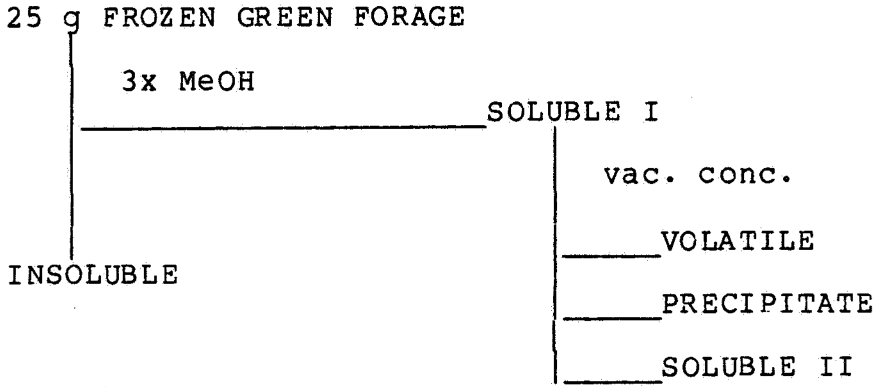
Green forage samples were extracted with three solvents, diethyl ether, methanol, and 50 percent methanol. Diethyl ether extracted only 8 percent of the total ^{14}C residue; therefore, these extracts were not examined further.

Methanol extracted about 40 percent of the total green forage ^{14}C activity. These extractions and subsequent separations are summarized by the registrant below:

Table 3

Separations Performed on Green Forage Samples and Control Samples Spiked with Reference ^{14}C-Labeled Compounds

OUTLINE:



(Cont'd next page)

Table 3

Separation Performed on Plant Samples, and Control Samples Spiked with Reference ^{14}C-Labeled Compound (Cont'd)

DATA SUMMARY

Sample	Insol.	Soluble I	Vol.	Ppt.	Soluble II
	Percent of Initial ^{14}C Activity				
Planting #1 (3624011 DPM)	49.4	48.4	1.6	2.7	45.4
Planting #3 (1282100 DPM)	59.7	42.8	1.9	3.5	37.3
Planting #3[a] (1254225 DPM)	60.8	44.7	1.8	3.9	37.1
Control[b] + ^{14}C-1,3-D (1034596 DPM)	0.2	116	103	0.0	0.4
Control[b] + ^{14}C-CAA (3498140 DPM)	0.1	94.7	18.2	0.5	76.1

---

[a] Performed 23 weeks after the initial analysis.
[b] Lettuce foliage from lettuce metabolism study.

According to the registrant, the data in Table 3 indicate that 1,3-D and CAA are not present in the green forage residues and suggest that much, if not all, of the ^{14}C activity is naturally incorporated. Also, a further search for direct metabolites of 1,3-D in these samples by measuring the dry weight concentration of ^{14}C in each fraction (i.e., searching for a "hot" fraction) was carried out. The results are outlined in Table 4, on the following page.

According to the registrant, his separation included an acid hydrolysis of the methanol-insoluble fraction to look for bound residues, and a separation by polarity (using a C_{18} Sep-Pak) of the major soluble fraction into two fractions. Only one fraction, the methanol eluate of the Sep-Pak separation, contained an outstanding concentration of ^{14}C activity.

Table 4

Separations Performed on Green Forage, Planting #3

OUTLINE:

- 25 g FROZEN GREEN FORAGE (% OF ORIGINAL ^{14}C ACTIVITY, 1254225 DPM)
  - 3x MeOH
    - SOLUBLE I (44.7%)
      - vac. conc.
        - VOLATILE (1.8%)
        - PRECIPITATE (3.9%)
        - SOLUBLE II (37.1%)
          - C_{18} Sep-PAK
            - H_2O ELUATE (15.3%)
            - MeOH ELUATE (21.8%)
    - INSOLUBLE I (60.8%)
      - HCl, 100 °C
        - SOLUBLE III (40.7%)
        - INSOLUBLE II (18.7%)

^{14}C Concentration as ppm 1,3-D equivalent Dry Basis

Green forage	15.0
Insoluble I	11.5
Insoluble II	8.1
Precipitate	11.3
Soluble II	30.8
H_2O eluate	16.6
MeOH eluate	115.5
Soluble III	9.9

HPLC analysis performed on the Soluble III fraction (acid hydrolyzate) revealed only very polar material eluting at the apparent void volume. HPLC of the water eluate fraction of the Sep-Pak separation revealed primarily very polar material and one component of intermediate polarity. The registrant concludes that these fractions probably contain naturally incorporated products; for example, sugars and amino acids which are not retained by the C_{18} column under these development conditions. HPLC analysis performed on the methanol eluate fraction of the Sep-Pak separation indicated the presence of two major and several minor components of intermediate polarity. These components did not cochromatograph with the direct metabolites of 1,3-D (i.e., CAA and 3-chloroacrylic acid) and according to the registrant suggest tertiary degradation products.

According to the registrant, these tertiary degradation products (metabolites) represent approximatley 27 and 29 percent of the total ^{14}C residues in plantings #1 and #3, respectively.

Characterization of the Bean Residues

Steam distillation of the bean sample yielded only 0.3 percent of total ^{14}C activity as volatile. The registrant states that the extraction and separation steps used with the bean sample were designed to isolate relatively pure natural components in order to confirm the presence of naturally incorporated ^{14}C activity, as well as to search for direct metabolites of 1,3-D. The results of most of these steps are outlined in Table 5 on the following page.

The regisrant explains that the ^{14}C concentration data given in Table 5 indicated that all fractions contained about the same level of ^{14}C activity. HPLC analyses of the water-soluble fraction (Soluble III) and the fraction solubilized by acid hydrolysis (Soluble IV) revealed the presence of only polar material. Additional protein was examined further, as shown in Table 6, on page 29.

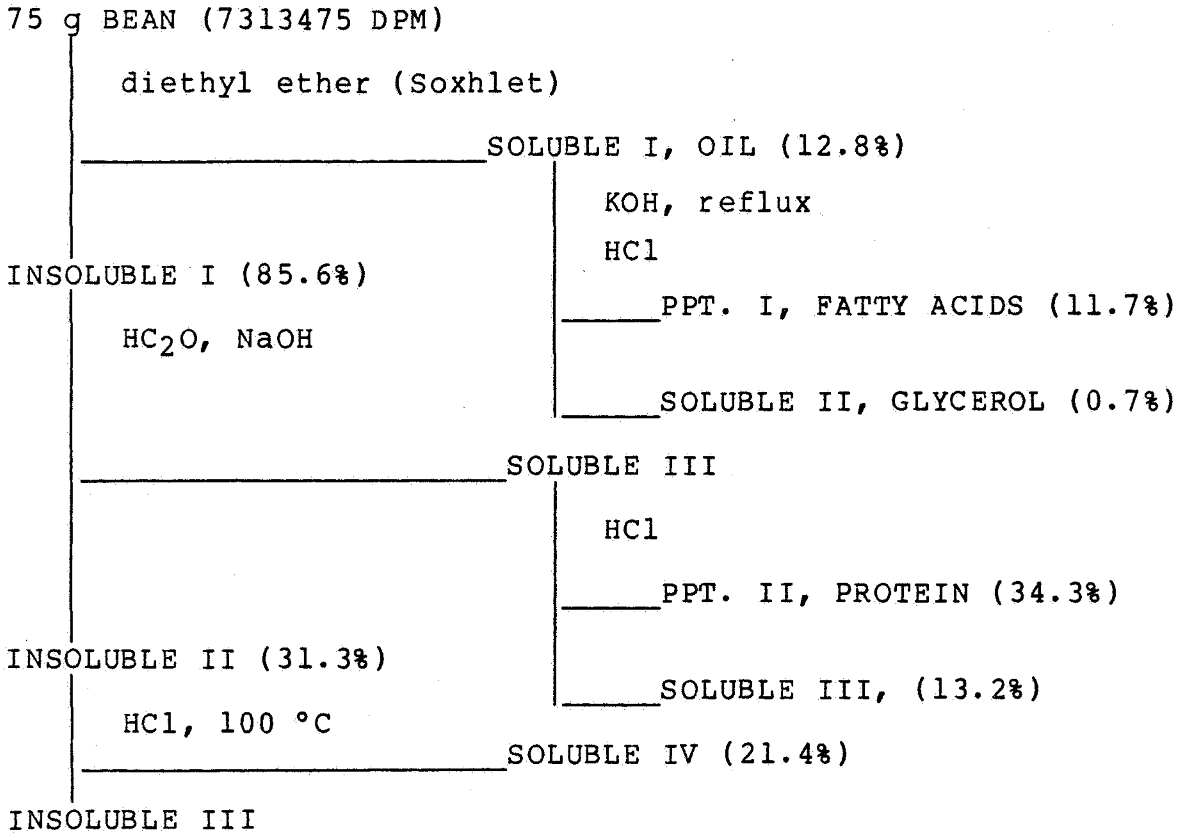
The registrant states:

> The protein was hydrolyzed by refluxing in a 7 N HCl for 24 hr, and glutamic acid hydrochloride obtained by cold precipitation (9). The free amino acid was obtained by isoelectric precipitation at pH 4.4. There was no significant loss of ^{14}C activity from protein to amino acid, again confirming natural incorporation. The identity of the glutamic acid

Table 5

Separations Performed on the Mature Soybean

OUTLINE:



^{14}C Concentration as ppm 1,3-D Equivalent Dry Basis

Whole Bean	5.6
Insoluble I	6.2
Insoluble II	5.0
Insoluble III	2.5
Soluble I, Oil	3.6
Precipitate I, Fatty Acids	3.5
Soluble II, Glycerol	1.7
Precipitate II, Protein	9.2
Soluble III	4.1

Table 6

Hydrolysis of the Bean Protein and Isolation of Glutamic Acid

OUTLINE:

10 g PROTEIN (1650720 DPM)

HCl, reflux
activ. C, filter
conc., HCl, cool

SOLUBLE I, (90.2%)

PRECIPITATE I, GLU. A. HCl (1.8%)

NaOH, ethanol

SOLUBLE II (0.6%)

PRECIPITATE II, GLU. A. (1.2%)

^{14}C Concentration as ppm 1,3-D Equivalent Dry Basis

Protein	9.2
Soluble I	6.1
Precipitate I, Glu. A. HCl	5.4
Precipitate II, Glu. A.	6.0

> as the N-trifluoroacetyl methyl ester was confirmed by GC-MS.
>
> To compare the bean residue with the green forage residue a sample of the defatted bean was extracted with 50 percent methanol. About 19 percent of the ^{14}C activity was solubilized. An HPLC chromatogram of this extract is shown in Figure 19. It is concluded that the metabolic products observed in the green forage samples were not translocated to the bean and that all of the bean residue is due to natural incorporation (10).

RCB's Conclusion

RCB does not agree with the registrant that all of the ^{14}C bean residue is due to natural incorporation. The registrant did not 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. In some cases, significant quantities of solubilized ^{14}C residues were obtained but no attempt was made to characterize these residues (for example, see Figures 8, 14, and 24 of the soybean metabolism study).

Characterization of Field Trash Residues

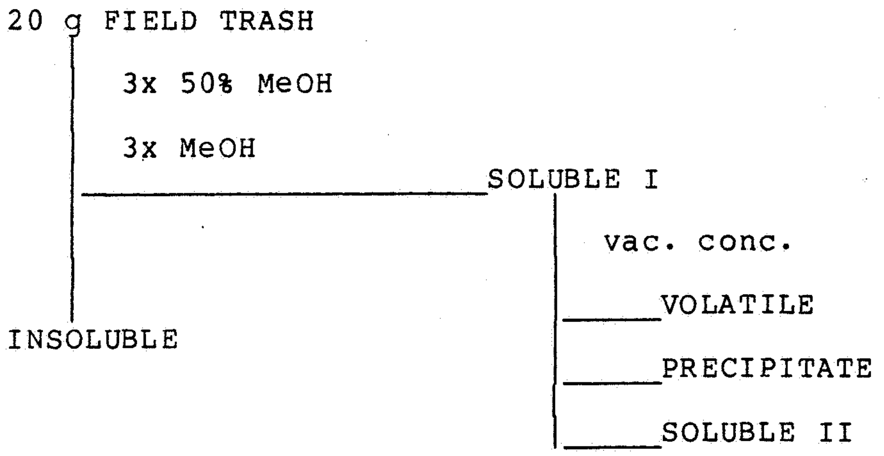
Steam distillates of the field trash sample yielded only 3.2 percent of the total activity as volatile. According to the registrant:

> Extraction of trash with diethyl ether solubilized only 4.6 percent of the ^{14}C residue. Extraction with methanol solubilized 29.1 percent of the residue and HPLC analysis of this extract indicated a residue pattern similar to the green forage samples (i.e., the presence of very polar material eluting at the apparent void volume and several components of intermediate polarity).
>
> More of the trash residue was solubilized with 50 percent methanol. The results of the intiial and a later extraction, and a recovery run are given in Figure 21 [Table 7].

Table 7

Separations Performed on the Field Trash Sample and Spiked with Reference ^{14}C-Labeled Compound

OUTLINE:



DATA SUMMARY:

	Percent of Initial ^{14}C Activity				
Sample	Insol.	Soluble I	Vol.	Ppt.	Soluble II
Initial Run (1922380 DPM)	56.5	47.3	2.7	44.6	44.6
Later Run[a] (1984500 DPM)	53.4	44.6	1.9	2.2	40.7
Control Trash + ^{14}C-CAA (3496428 DPM)	0.5	101.3	14.9	2.1	75.9

---

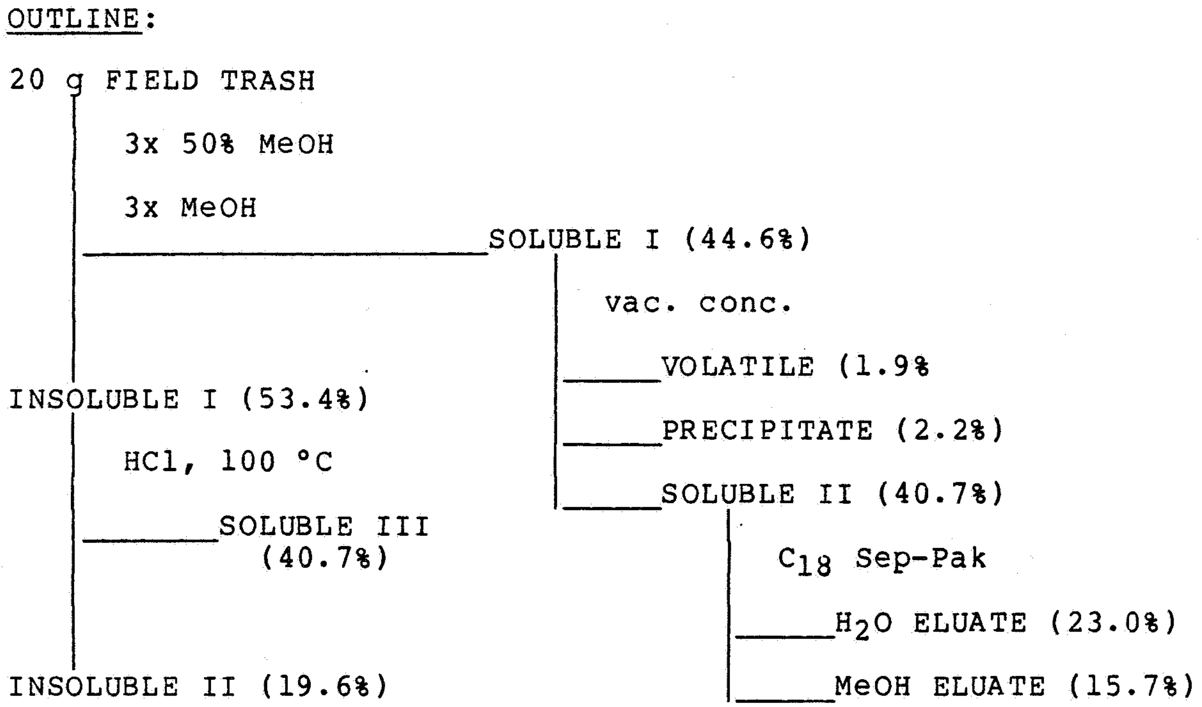
[a] Performed 15 weeks after the initial analysis.

An HPLC chromatogram of the Soluble II fraction revealed a major peak of intermediate polarity eluting at 24 min which represented 8 percent of the total field trash ^{14}C residue. In this chromatogram, summation of all the peaks beyond the void volume represented about 20 percent of the total ^{14}C residue in the field trash.

In an attempt to search for a fraction containing the highest concentration of ^{14}C activity, the registrant performed the separations on field trash outlined in Table 8.

Table 8

Separations Performed on Field Trash



^{14}C Concentration as ppm 1,3-D Equivalent, Dry Basis

Field Trash	5.8
Insoluble I	3.5
Precipitate	9.1
Soluble II	17.7
H_2O Eluate	8.8
MeOH Eluate	65.7
Soluble III	3.8
Insoluble II	3.4

As was the case with soybean green forage, the methanol eluate of the Sep-Pak separation was the fraction containing the highest concentration of ^{14}C activity. HPLC analysis performed on this fraction indicated the presence of two major and two minor components of intermediate polarity. The registrant noted a similarity of the ^{14}C elution pattern in this HPLC histogram to the corresponding soybean green forage fraction. These ^{14}C components did not cochromatograph with the direct metabolites of 1,3-D (i.e., CAA and 3-chloroacrylic acid) with the largest peak representing about 7 percent of the total ^{14}C trash residue. HPLC analyses performed on both the Soluble III fraction (acid hydrolyzate) and the water eluate fraction of the Sep-Pak separation yielded only very polar ^{14}C material.

According to the registrant, the metabolism data indicate that the ^{14}C residue in field trash samples consists primarily of incorporated ^{14}C activity including small amounts of possible metabolic products. In addition, there appears to be no major change in the character of the residue from the green forage stage to the fully mature field trash stage.

RCB's Conclusion

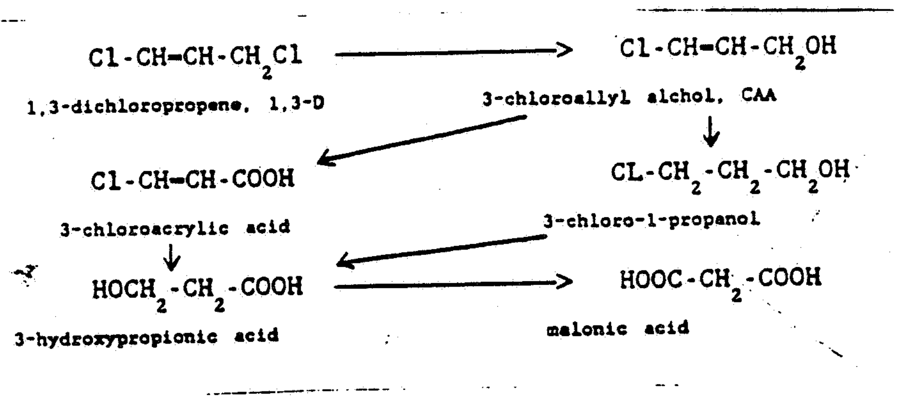
RCB does not agree with the registrant that field trash samples consist primarily of incorporated ^{14}C activity or that there is no change in the character of the residue from the green forage stage to the fully mature field trash stage.

The registrant did not use exhaustive Soxhlet extraction, different strengths of acid and/or base, and enzymatic techniques in order to release conjugated/bound residues in the insoluble fractions. In some cases, significant quantities of ^{14}C residues were released but no attempt was made to characterize these residues (see Figures 8, 14, and 24 of the soybean metabolism studies).

Characterization of the Soil Residues

Soil residues of 1,3-D were previously characterized in conjunction with the lettuce and spinach ^{14}C metabolism study conducted at the same field location.

The registrant also proposed a possible metabolic pathway of 1,3-D to natural products in plants as follows:



Attachment - Pesticide Assessment Guidelines, Subdivision O, Addendum on Data Reporting

cc: With Attachment: Reviewer(M. Kovacs), TOX, Registration Standard (Telone), RF, E. Eldredge (ISB/PMSD).

cc: Without Attachment: Circulation (7), SF (Telone).

RDI:J.Onley; 6/21/88: R.D.Schmitt; 6/24/88

TS-769C:RCB:CM#2:Rm 814:x7689:Typist Kendrick: edited by mk 7/1/88

Attachment
to ID No. 464-511-
Telone Review
RCB No. 3654

PESTICIDE ASSESSMENT GUIDELINES

SUBDIVISION O

HAZARD EVALUATION:

RESIDUE CHEMISTRY

Series 171-4 (a)(1)&(2)

Nature of the Residue: Plants

ADDENDUM ON DATA REPORTING

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Subdivision O - Nature of the Residue: Plants

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PESTICIDE ASSESSMENT GUIDELINES

RESIDUE CHEMISTRY

Nature of the Residue: Plants

Subdivision O, Series 171-4

DATA REPORTING

INTRODUCTION

A. Purpose

Plant metabolism studies which provide residue chemistry data on the qualitative nature of the residue 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 (FIFRA).

Subdivision O [§§ 171-4(a)(1) and (2)] of the EPA Pesticide Assessment Guidelines provides information to aid the petitioner/registrant in conducting plant metabolism studies. A metabolism study must be submitted for each type of plant for which use is proposed and in general, one metabolism study will be required for each of the crop groups defined in 40 CFR 180.34(f), except for herbs and spices. However, if the results of three metabolism studies on dissimilar crops (e.g., root crop, oilseed and a leafy vegetable) indicate a similar metabolic route in the three crops, then additional plant metabolism studies in other crops for which use is proposed will not be required.

The Data Reporting Guideline is designed to aid the petitioner/registrant in the data/information collection/organization process and thereby facilitate the Agency review process. Data submitters are encouraged to submit complete reports following this guidance for efficient review by the Agency. This guidance pertains to the substance of the data report. PR Notice 86-5, effective on November 1, 1986 (available from the Registration Support and Emergency Response Branch, Office of Pesticide Programs, US EPA) pertains to the physical formating of reports (which are refered to as "studies") and submittal packages. Some of the requirements in PR Notice 86-5 are mandatory.

B. Objective

Plant metabolism studies should characterize the qualitative nature of the total terminal residue and thereby facilitate a chemical definition of the "total toxic residue" in or on the raw agricultural food or feed commodity resulting from application of the pesticide formulated product to crops. These studies are to answer the question, <u>what</u> is the chemical residue?

The term total terminal residue is used to refer to all components of the resultant residue whether or not they are toxic enough to be of concern. Based on the results of the residue characterization studies described in the plant metabolism submission, the petitioner/registrant should propose the chemical definition of the "total toxic residue." The term "total toxic residue" is defined as the sum of the parent pesticide and its degradation products, metabolities (free or bound), and impurities that are considered to be of toxicological significance, and therefore warrant regulation. All components of the "total toxic residue" must be included in the analysis of the magnitude of the residue for samples used to quantitate the level of residue likely to occur.

If, after exhaustive attempts, the petitioner/registrant is unable to arrive at a determination as to whether the total terminal residue has been sufficiently characterized, which metabolities should be covered by the tolerances, and which components of the residue must be determined by the residue analytical methodology, then these written and documented concerns should be expeditiously communicated to EPA staff in order to arrive at their mutually acceptable and timely resolution prior to the proposed final definition of the "total toxic residue."

RESPONSE TO PUBLIC COMMENTS

The purpose of this section is to acknowledge and address the concerns expressed in the letters of comment received by the Agency in response to the public notice in FEDERAL REGISTER Volume 51, No. 98, pp. 18660-18661, May 21, 1986.

This addendum to the Pesticide Assessment Guidelines [Subdivision O] is to be considered an all-encompassing document. EPA recognizes there are sections in the addendum which do not apply in all cases. Therefore, registrants should exercise scientific judgement in deciding which portions are germane to a specific data submission.

This Data Reporting Guideline (DRG) is not intended to introduce new data requirements or revisions into the Pesticide Assessment Guidelines [Subdivision O] nor is it intended for use by Agency data reviewers as a mere checklist. It is intended to clarify ambiguities in interpretation of those existing Guidelines, and to organize the submission of data to facilitate the review process.

The Residue Chemistry Branch (RCB) has reviewed the nine comments submitted by the registrants and/or committees in regard to the Data Reporting Guideline. All of the comments submitted were study-specific and were addressed as follows:

1. <u>Comment</u> - The appropriateness of the inclusion of the statement regarding the capability of the proposed enforcement analytical method(s) to determine the major components of the terminal residue and all components of the total toxic residue was questioned.

   <u>Response</u> - Under the present guidelines §171-4(a)(1), this statement is required. However, in response to these comments, the statement was modified throughout this DRG and, in addition, a note was made that if the specified statement/information is provided elsewhere within the

overall data submission package, it need not be reiterated in the section on plant metabolism but referenced.

2. Comment - The reporting requirements for dosage rates, quantitation and distribution of total recovered radioactivity, minor metabolites and degradation routes or pathways of metabolism need to be clarified.

Response - Revisions were made to the DRG in response to those comments whenever possible.

3. Comment - The certification requirement is unnecessary since personnel responsible for conducting the study sign the report and maintain records on the study.

Response - The requirement is retained in the DRG since it is consistent with the same requirement previously proposed in the DRGs for Magnitude of the Residue: Crop Field Trials and Analytical Method(s).

GUIDELINE

The petitioner/registrant's report on plant metabolism studies should include all information necessary to provide a complete and accurate description of treatments and procedures. The information submitted in the report should include the following elements:

1. Radiolabeling techniques to include rate, method, and time of radiolabel application in relation to the development and growth cycle of the treated raw agricultural commodity (r.a.c.);

2. Extraction, fractionation, and characterization techniques employed for the identification of residue components whether free or bound at each sampling interval;

3. Definition of total terminal residues, to include data for all major components of the total terminal residue reflecting their distribution within the r.a.c. (including processed fractions derived therefrom) expressed as both percentage of the total recovered radioactivity and concentration (parts per million [ppm]) found at time of harvest and/or when utilized for animal feed;

4. A detailed discussion, preferably accompanied by a flowsheet format, of the possible routes of degradation or pathways of metabolism observed in the subject r.a.c.;

5. When enforcement analytical methodology has been developed, these methods must be validated with radiolabeled samples derived from the plant metabolism study, accompanied by a statement made as to their capability to determine the identified major components of the terminal residue whether free or bound/conjugated, and all components of the total toxic residue whether free or bound/conjugated in the r.a.c. [See note under III.B.7.]

The following is the format for the report.

MASTER COVER PAGE

Title page and additional documentation requirements (i.e., requirements for data submission and procedures for claims of confidentiality of data) if relevant to the study reported should precede the content of the study formatted below. These current mandatory requirements are described in PR Notice 86-5.

MASTER TABLE OF CONTENTS AND INDEX

A concise listing, preceding the body of the report, of all essential elements of the study, and the page or table number where the element is located in the report to include the following:

A. Introduction and Summary

B. Materials

C. Methods (Experimental)

D. Results and Discussion

E. Conclusions

F. List of Tables

G. List of Figures (Flowsheets, etc.)

H. References

I. Appendices

I. MASTER INTRODUCTION/SUMMARY

A. General

Overall, this section should include appropriate background and historical information relative to the study. In addition, the purpose and overall summary of the study, a discussion of the results obtained, and conclusions arrived at regarding the qualitative nature of the total terminal residue in the treated crop should also be included in this section.

B. Specific

The following specific topics should be briefly discussed in this section:

1. Registration history and proposed use of the subject chemical.
2. If applicable and/or available via an appropriate citation or reference, compare and contrast observed metabolic routes in the subject r.a.c. to those observed in earlier plant metabolism studies conducted on

the subject r.a.c. or on other commodities or to those observed in animal metabolism studies conducted with the subject chemical.

3. The purpose of the study, to include testing strategies employed and the rationale for the selection of these strategies.

4. The overall experimental procedure employed, to include a discussion, if applicable, of unusual experimental problems encountered, attempts made to alleviate these problems which resulted in deviations from the intended test protocol and the effects, if any, of those deviations on the results of the study.

5. The modes and routes of metabolism observed including a complete description of the identity and quantity (both free and bound) of all major components of the terminal residue and their distribution within the r.a.c. and processed fractions derived therefrom. (The foregoing information could be summarized in a narrative and/or tabular, figure format.)

6. A conclusion concerning the qualitative nature of the terminal residue in the r.a.c. (including processed fractions derived therefrom) at time of harvest or when utilized for animal feed.

7. When enforcement analytical methodology has been developed, these methods must be validated with radiolabeled samples derived from the plant metabolism study, accompanied by a statement made as to their capability to determine the identified major components of the terminal residue, whether free or bound/conjugated and <u>all</u> components of the total toxic residue whether free or bound/conjugated in the r.a.c. The statement should also indicate the detection limits, precision, and accuracy of the methodology employed. [Note: If the specified statement/information is provided elsewhere within the overall data submission package, it need not be reiterated in this section, but referenced as to location in the data submission package.]

II. MATERIALS

A. Test Substance

In this section the following should be included:

1. Identification of the test pesticide active ingredient (ai), including chemical name; common name (ANSI, BSI, ISO); company developmental/experimental name; and Chemical Abstracts Service (CAS) number.

2. Chemical structure(s) for the parent compound and metabolites constituting the residue.

3. Information on relevant formulation parameters as pertinent; e.g., nature of the solvent, carrier, bait, adjuvant, or other matrix in which the radiolabeled pesticide was applied.

4. For radiolabeled test material, report its purity, specific activity in Curies/mole, disintegrations per minute per gram (dpm/g), nature of the radiolabel and its source and the site(s) of labeling in the molecule. The identity of radiolabeled impurities, if any, derived from the test material should also be reported.

5. A rationale provided for selection of radiolabels other than 14C and for site(s) of labeling in the molecule (where possible, emphasis is placed on labeling the ring position).

6. Other - Any and all additional information the petitioner/registrant considers appropriate and relevant to provide a complete and thorough description of the test chemical, such as physical-chemical properties (e.g., solubility, etc.).

B. Test Site

In this section the following should be included:

1. A detailed description of the overall testing environment utilized for the study (i.e., outdoor test plots, greenhouse, or plant growth chambers) including, as appropriate, a record of environmental conditions experienced during the course of the study (i.e., temperature, rainfall, sunlight) and documentation of soil characteristics at the testing site.

2. An explanation or rationale provided by the petitioner/registrant if the reported testing environment, including testing media, employed in the metabolism study is not representative of or differs significantly from expected cultural practices or environmental conditions under which the test crop would normally be grown.

C. Test Crop

In this section the following should be included:

1. Identification of the test crop including type/variety and crop group classification according to 40 CFR 180.34(f), as revised June 29, 1983; 48 FR 29855;

2. A rationale or statement provided by the petitioner/registrant for selection of a test crop other than that for which use is proposed.

3. Identification of specific crop part(s) harvested and subjected to 14C residue analysis for a determination of the "total terminal residue."

4. The developmental stage(s), general condition (immature/mature, green/ripe, fresh/dry, etc.) and size(s) of the test crop at time of pesticide application(s) and at harvesting(s).

5. Other - Any and all additional information the petitioner/registrant considers appropriate and relevant to provide a complete and thorough description of the test crop.

III. METHODS

A. Application of the Pesticide

In this section, the following should be included:

1. A detailed description of the type of pesticide application(s) to the test crop (i.e., preplant soil incorporated, over-the-top post-emergent foliar application, bait application, etc.), including the formulation (i.e., solvent, carrier, bait, adjuvant, or other matrix) in which the radiolabeled pesticide was applied and the method of application (i.e., hand sprayer, topical, soil injection, etc.).
2. The actual dosage rate(s) (i.e., mg/kg or ppm of plant or soil treated and expressed as kg ai/ha or lbs ai/acre where possible) used in the study.
3. Number and timing of application(s), between-application interval(s), and treatment to sampling interval(s) (also known as TSI, or PHI).
4. Dates [planting/sowing/transplanting, as applicable; other significant dates in the growing of the crop (e.g., harvesting of immature crop to obtain specific crop part(s) which may be utilized for animal feed); pesticide application(s); harvest of mature crop].
5. An explanation or rationale by the petitioner/registrant for any significant deviation in either the rate or mode of application to the test crop from the intended use pattern.

B. Sample Harvest (Collection)

1. Harvest procedures (method of harvesting or collection [mechanical/hand; from the plant/ground/flotation; etc.]; type of equipment used; number/weight of samples collected per replication and number of replications per treatment level; sample coding/labeling). These procedures should clearly state the sampling procedure used to obtain representative samples.
2. A detailed description of additional relevant information on the growing of the test crop, application(s) of the pesticide formulated product(s), and harvesting(s) of samples.

Refer to the Data Reporting Guideline on Magnitude of the Residue: Crop Field Trials[1] for additional guidance on this subject area.

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[1] Published by NTIS, Document # PB86-248192.

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C. Sample Handling and Storage Stability

1. A detailed description of the handling, preshipping storage, and shipping procedures, as applicable, for harvested (collected) samples.

Refer to the Data Reporting Guidelines on Magnitude of the Residue: Crop Field Trials[1] for additional guidance on this subject area.

2. A detailed description of the conditions and length of storage of harvested (collected) samples following their receipt in the laboratory.

Refer to the Data Reporting Guidelines on Storage Stability Study[2] for additional guidance on this subject area.

D. Analyses of Radioactive Residues

1. Quantitation and distribution of total recovered radioactivity

    a. Total recovered (i.e., combustible) radioactivity remaining on the plant at time of sampling or harvest should be reported.

    b. Quantitative radioactivity data reported for all plant parts sampled, including fractions thereof which may be processed into food or feed, at time of normal harvest or at a stage of development when normally utilized for animal feed.

    c. A detailed description of sample preparation (i.e., dissection, grinding, lyophilization, etc.) prior to oxidative combustion/liquid scintillation analyses.

    d. A quantitative accountability for a majority of total radioactivity recovered from the treated crop at times of sampling or harvest as a result of aggregate sample analyses performed above in item (1)(c).

    e. A detailed description provided in narrative, figure, or tabular format of total distribution of radioactivity in the treated crop (and processed fractions derived therefrom) at time of sampling or harvest.

    f. Details of analytical method parameters including descriptions of equipment used for determining total radioactivity in each sample should be furnished.

    g. Details of radioactive counting data for selected representative samples to include counting times, total counts recorded, corrected counts, counting efficiencies, other raw data (sample

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[1,2] Published by NTIS, Document # PB86-248192.

sizes, ppm equivalents found, sensitivity, limit of detection) including representative calculations should be reported.

h. For each sample analyzed (plant part or fraction) results should be reported as: (1) total radioactive counts (dpm/ug); (2) the percentage that these radioactive counts represent of the total recovered radioactivity in the treated plant at time of sampling or harvest; and (3) the ppm equivalents (expressed as parent compound) that these radioactive counts represent of the total recovered radioactivity in the treated plant at time of sampling or harvest.

2. Extraction and Fractionation of Radioactivity

a. A complete description, preferably accompanied by a flowsheet or diagram depicting the overall extraction and fractionation strategies (schema) employed for each sample matrix analyzed.

b. A discussion of and rationale for the selection and extraction sequence for extracting solvent (polar vs nonpolar) used and extraction procedures (i.e., blending, maceration, partitioning, soxhlet) employed, including use of additional techniques (i.e., decomplexing reagents, ultrasonics, etc.) should be provided.

c. A description of conditions employed for the acidic, basic and/or enzymatic hydrolysis of (the filter cake or residue remaining from) previously extracted plant tissue and/or water soluble plant extracts to release conjugated residues from these samples. Specific information on the source, purity, specificity, and activity of all enzymatic preparations utilized for hydrolysis should also be provided.

d. Calculations provided showing the ratio and/or amounts of total free vs conjugated parent compound and/or metabolites in each extracted sample matrix.

e. The petitioner/registrant should provide a quantitative estimate of residual radioactivity (i.e., "unextractable or bound") remaining in the extracted sample matrix following both exhaustive solvent extractions and hydrolytic treatments. The residual radioactivity reported should be expressed as both percentage and ppm (as parent equivalents) of total recovered radioactivity. Attempts at "bound residue" extraction by exotic or other procedures, or extractions following repeated treatments with concentrated acids and/or bases at elevated temperatures should also be reported by the petitioner/registrant and a rationale for their use given.

f. Radiochemical extraction efficiencies calculated and reported for all harvested plant tissues.

g. The efficiency of separation and purification for all fractionation and isolation techniques employed in the study (i.e., solvent partitioning, high voltage electrophoresis, ion-exchange, or exclusion column chromatography, HPLC using gradient elution, 2-dimensional thin-layer radioautography employing multiple solvent systems) should be reported for a representative sample.

h. Data to account for or track loss of radioactivity in each subsequent step of the fractionation and isolation procedure should be provided and attempts made by the petitioner/registrant to minimize these losses should be discussed.

i. The petitioner/registrant should report detailed procedures for the fractionation of "unextractable or bound" radioactivity in plant tissues into proteins, starch, lignin, cellulose, etc., <u>only if</u> these residues, as reported in item (2)(e) above, are located in edible plant parts used for food or animal feed <u>and</u> their total concentration is generally greater than 10 percent of the total radioactivity recovered from the treated plant <u>or</u> when normalized to use-conditions generally greater than 0.1 ppm (expressed as parent equivalents).

j. Following chemical analyses of the fractionated plant tissues described above in item (2)(i) for amino acids, glucose, etc., the petitioner/registrant should then report if significant quantities of the original radioactive residue characterized as "unextractable or bound" have been incorporated into these natural products.

k. The amount of radioactivity in each sample fraction (e.g., water soluble, organosoluble, released by hydrolysis, etc.) should be quantified and reported in terms of total radioactive counts, and as both percentage and ppm (as parent equivalents) of total radioactivity recovered in the original sample matrix analyzed.

3. <u>Characterization of Radioactivity</u>

a. A complete tabular listing and description of all known and suspected metabolites of the parent compound (model compounds, including their structure and purity) used to facilitate the characterization and/or identification of unknown sample metabolites should be provided.

b. Calculations should be provided and data reported for both sample and reference R_f values on TLC radioautograms and for relative retention times on GC and HPLC columns. Unexpected deviations or variances of observed from expected values including loss of sample resolution between analytes in subsequent chromatographic analyses should be reported and steps taken to correct these problems should be discussed.

c. The petitioner/registrant should also provide complete details of additional confirmatory analytical procedures used to separate and characterize metabolites [i.e., high voltage electrophoresis, ion-exchange, or exclusion chromatography, derivatization, etc.] and determinative methods (i.e., Mass Spectroscopy in EI and CI modes) used for ultimate identification of metabolite(s).

d. All lost or unaccounted for radioactivity in each plant extract or fraction should be explained as fully as possible and the amount reported should be expressed as both percentage and ppm (as parent equivalents) of total radioactivity recovered from the particular plant part or fraction analyzed and of the total plant at harvest (terminal residue) or when utilized as an animal feed.

e. Individual and/or aggregate quantitative radioactive residue data provided for all non-identified and/or non-characterized discrete extractable and resolvable radioactive entities with amounts reported as in item 3(d) above.

f. The petitioner/registrant should report each of the major metabolite components (i.e., those generally comprising ≥ 10 percent of the total recovered radioactive residue) and, if possible, provide information on the chemical nature of discrete (minor) metabolite components. Major metabolite components should be quantified with amounts reported as in item 3(d) above; quantification of minor metabolite components should be attempted and the results reported, if possible.

g. The petitioner/registrant should report data/information delineating attempts made to chemically characterize conjugated or complex bound chemical species originating from the parent pesticide only if significant quantities of these "unextractable or bound" residues are present in edible plant parts used for food or animal feed (see testing criteria described in item (2)(i) above) and if the results of the fractionation/characterization study described above in item (2)(j) also indicated that significant levels of incorporation of these residues into natural plant products (i.e., amino acids, glucose, lignin, etc.) did not occur.

h. Quantitative data (with amounts reported as in item 3(d) above) should also be reported by the petitioner/registrant for each minor metabolite component identified in item (3)(f) above if this minor metabolite is also identified as a discrete extractable and identifiable component of the bound residues chemically characterized in item (3)(g) above and its total concentration (free and bound) generally comprised ≥ 10 percent of the total recovered radioactive residue.

i. When enforcement analytical methodology has been developed, the petitioner/registrant must report the results of analysis by these methods on radiolabeled samples derived from the plant metabolism study. The analysis should specify what percentages of the total radioactivity and of each labeled and identified major component of the terminal residue whether free or bound/conjugated in/on the treated crop at time of sampling or harvest are accounted for by these methods. [See note under III.B.7.]

j. A complete description of all instrumentation, equipment, and reagents used, including operating conditions of the instrumentation utilized for the separation, characterization, and identification of radioactive residues should be submitted. Photographs of radio TLC plates as well as samples or reproductions of HPLC/GLC chromatograms including mass spectral scans, etc., should also be submitted.

E. Other - Any and all additional information the petitioner/registrant considers appropriate and relevant to provide a complete and thorough description of the conduct of the plant metabolism study and the determination of the total terminal residue.

IV. RESULTS AND DISCUSSION

A. Test Strategies

This should include a discussion of deviations made from the intended testing protocols or strategies as a result of unusual experimental problems or conditions encountered in growing, treating, or sampling the test crop to include difficulties in extraction, fractionation, and characterization of residues and, if applicable, specific extraction and characterization strategies employed for unextractable or bound residues; including a discussion of the impact or effects, if any, of those deviations on the results of the study.

B. Metabolic Pathways

If possible, a detailed discussion, preferably accompanied by a flowsheet format, of the routes of degradation or pathways of metabolism observed in the subject r.a.c. should be provided. For discussion purposes, the observed metabolic routes in the subject r.a.c. may be compared and contrasted to known and previously reported metabolic pathways in other r.a.c.s or observed in animal metabolism studies conducted with the subject chemical.

C. Characterization and Distribution of Total Terminal Residue

1. In a tabular or graphic format, identification [including name, structure, and quantity (expressed both as percentage and ppm as parent equivalents)] of all major components of terminal residue in r.a.c. (both free and conjugated/bound) and their distribution

within the r.a.c. including plant parts and processed fractions derived therefrom.

2. If the immature r.a.c.(including plant parts and processed fractions thereof) is normally utilized for animal feed, then identification and quantification of all major components of the residue present at that stage of plant development must also be reported as in item (C)(1) above.

3. Provide information on any properties and/or characteristics of, their quantities, and distribution within the r.a.c. of all significant unidentifiable and/or uncharacterizable components of the terminal residue.

4. When enforcement analytical methodology has been developed, the petitioner/registrant must report the results of analysis by these methods on radiolabeled samples derived from the plant metabolism study. The analysis should specify what percentages of the total recovered radioactivity and of each labeled and identified major component of the terminal residue whether free or bound/conjugated in/on the treated crop at time of sampling or harvest are accounted for by these methods. [See note under III.B.7.]

D. Residue Analytical Methodology

This should include a discussion of the efficiency of extraction procedures employed for all harvested plant tissues and quality assurance measures/precautions taken to ensure the integrity of all stored samples prior to analysis. When enforcement analytical methodology has been developed, these methods must be validated with radiolabeled samples derived from the plant metabolism study accompanied by a statement made by the registrant/petitioner as to the capability of these methods to determine the identified major components of the terminal residue whether free or bound/conjugated and all components of the total toxic residue whether free or bound/conjugated in the r.a.c. [See note under III.B.7.]

E. Statistical Treatment(s)

If during the course of the plant metabolism study, statistical tests are applied to the raw data obtained during sampling/analyses, then representative examples of these tests should be described.

F. Other - Any and all additional information the petitioner/registrant considers appropriate and relevant to provide a complete and thorough description of the plant metabolism study including quality control measures/precautions taken to ensure validity of all aspects of the study.

V. CONCLUSIONS

Discuss conclusions that may be arrived at as a result of the submitted plant metabolism study, such as:

A. The routes or pathways, mechanisms involved and extent or degree of metabolism observed when the subject r.a.c. is grown to maturity or harvest.

B. The nature, amount, and distribution of the total terminal residue in the r.a.c. (including processed fractions derived therefrom at time of harvest or when normally utilized for animal feed) resulting from the proposed use of the pesticide.

C. Based on the results of validation studies conducted on radiolabeled plant samples, the capability of developed and available enforcement analytical methodology to determine the identified components of the terminal residue whether free or bound/conjugated, and the capability of the same or modified analytical methodology to determine <u>all</u> components of the total toxic residue whether free or bound/conjugated in the r.a.c. [<u>See</u> note under III.B.7.]

VI. TABLES/FIGURES

A. <u>Tables</u> [examples given are]:

1. Tables of weather and/or environmental data.

2. Distribution and quantity of radioactivity in various harvested plant parts.

3. Name, structure, purity, for all (model compounds) metabolites utilized in study.

4. HPLC/GLC retention times and TLC R_f values for parent compound, metabolites, related compounds and model compounds under different column, solvent (elution) conditions.

5. Name, structure, quantity and location in r.a.c. of all major identified components of terminal residue.

6. Properties, characteristics, quantities and distribution within r.a.c. of all significant unidentified components of the terminal residue.

B. <u>Figures</u> [examples given are]:

1. Discussion or diagram of location, topography, and size of outdoor test plot(s).

2. Photographs, figures, or diagram of greenhouse and/or plant growth chamber facilities used in study.

3. Overall extraction and fractionation strategies or schema employed for each sample matrix analyzed.

4. Distribution of radioactivity in various ion exchange (exclusion) or preparative HPLC/GLC fractions.

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5. Metabolism flow diagrams or charts.

VII. CERTIFICATION

A signed and dated certification of authenticity by, and identifying information (typed name, title, affiliation, address, telephone number) on, the personnel responsible for the various phases of this report (e.g., Study Director, Field Supervisor, and Laboratory Supervisor).

VIII. REFERENCES

IX. APPENDIX(ES)

A. Representative chromatograms, spectra, etc. [as applicable].

B. Cite or reference reprints of published and unpublished literature, company reports, letters, analytical methodology, etc., used by the petitioner/registrant [unless physically located elsewhere in the overall data report, in which case cross-referencing will suffice].

C. Other - Any relevant material not fitting in any of the other sections of this report.