Endosulfan List A file

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SCIENTIFIC DATA REVIEWS
EPA SERIES 361

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Endosulfan (079401), Reregistration Case No. 0014 and Special Review. Registrant AgrEvo USA Company. Guideline 860.1300. Metabolism in Crops: Apple (MRID 44082701), Lettuce (MRID 44082702), and Cucumber (MRID 44099101), and Metabolism in Livestock: Cow (MRID 44082703) and Hen (MRID 44099102). CBRS No. 17547, DPBarcode No. D229661 (Crops), CBRS No. 17855, DPBarcode No. D234815 (Livestock).

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In support of reregistration, Registrant AgrEvo USA Company has submitted data on metabolism in crops and livestock. Assignment instructions are to review the submissions. Conclusions and Recommendations below pertain only to the present submission.

Tolerances are established for total residues of the insecticide endosulfan, 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide, and its metabolite endosulfan sulfate, 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3,3-dioxide, in or on numerous commodities of crops and livestock (40 CFR 180.182 and 185.2600); structures of parent and the sulfate are included in Figure 1. Endosulfan is a List A chemical. A Reregistration Standard (Guidance Document) was issued April 1982. The Residue Chemistry Chapter was issued 9/17/81, and an Update to the Residue Chemistry Chapter was issued 8/9/90.

Conclusions

Conclusions 1 and 2 pertain to the metabolism study in lettuce:

1. In mature lettuce, 87% of total residues were identified. The large majority of residues consisted of the alpha and beta isomers of endosulfan; smaller amounts of endosulfan sulfate, and small amounts of endosulfan diol were also identified. Individual unidentified residues each represented less than 10% TRR. (See Figures 1 and 2 and Table 2).

2. Conditions for this study, two applications at 21 and 14 days pre-harvest, each at 1 lb ai/A, represented approximately the maximum rate for leaf lettuce. The nature of the residue in lettuce is adequately understood.

Conclusions 3 and 4 pertain to the metabolism study in cucumber:

3. In mature cucumber fruit, 50% of total residues were identified as alpha and beta endosulfan and endosulfan sulfate. In mature cucumber leaves, 69% of total residues were identified. Residues identified were the same as those in fruit, plus sugar conjugates of endosulfan diol and hydroxy endosulfan carboxylic acid; conjugates represented more than 30% of TRR in leaves. (See Figures 1 and 2 and Table 6).

4. Applications during this study represented half the maximum annual number, and approximately half the maximum annual rate. These conditions were not consistent with the requirements of the Update to the Residue Chemistry Chapter (8/9/90), which specified normal cropping conditions and exaggerated rates. However, the additional work on polar residues in leaves can be translated to fruit. With the data from leaves, the nature of the residue in cucumber is adequately understood.

Conclusions 5 through 7 pertain to the metabolism study in apples:

5. Application for this study represented approximately the maximum rate for the single application closest to harvest (21 day PHI). Use directions allow additional applications much earlier in the season, which would be expected to cause more extensive metabolism by harvest.

6. In apples harvested 21 days after application, 95% of total residues were identified. The large majority of residues in fruit consisted of the alpha and beta isomers of endosulfan; small amounts of the sulfate were also present. In 21 day leaves, 86% of total residues were identified. The sulfate represented nearly half the residues in leaves; alpha and beta endosulfan were also identified, along with small amounts of the diol. In fruit and leaves, individual unidentified residues each represented less than 10% TRR. (See Figures 1 and 2 and Table 9).

7. As Conclusion 5 indicates, a metabolism study with applications earlier in the season, consistent with registered use, would have been preferable for apples. However, if metabolism in apples were considerably more extensive than the submitted results indicate, the array of metabolites produced would be expected to be no greater than that observed with cucumber (Conclusion 3). With the combined data on apple fruit and leaves, the nature of the residue in apples is adequately understood.

Conclusions 8 through 11 pertain to the metabolism study in ruminants:

8. In milk, 89% of total residues were identified as endosulfan sulfate. In muscle, 66% of total residues were identified as alpha endosulfan and the sulfate. Although data were not required for heart, 46% of total residues in this organ were identified as alpha endosulfan, endosulfan sulfate, and endosulfan lactone. (Table 14 and Figure 6) In each of these tissues, individual unidentified residues each represented less than 10% TRR and/or less than 0.05 ppm.

9. In fat samples, 68% to 84% of total residues were identified as endosulfan sulfate (Table 14). Considering all the data from omental, renal, and subcutaneous fat, further work on these samples is not required.

10. In liver, 52% of total residues were identified as alpha endosulfan, endosulfan sulfate, and potential conjugates of endosulfan or specific metabolites. In kidney, 34% of total residues were identified as the sulfate and potential conjugates of endosulfan or specific metabolites. (see Table 13 and Figure 6) In liver and kidney, 30-50% of total residues were designated as polar compounds, but this assignment followed extensive attempts to cleave metabolites that may have been conjugated. With these considerations and the extensive identification of residues in milk, muscle, and fat, further work on liver and kidney is not required.

11. All of the metabolites identified contained intact chlorinated rings, including all labeled carbons, even after harsh treatments to cleave conjugates. Identified metabolites represented 50% TRR in liver and higher portions in fat, muscle, and milk (89% TRR). This information indicates the stability of labeled rings. With this consideration, and Conclusions 8 through 10 above, the nature of the residue in cow is adequately understood.

Conclusions 12 and 13 pertain to the metabolism study in hens:

12. Residues identified in poultry tissues ranged from 50% TRR in muscle to 95% TRR in fat; residues identified included alpha and beta endosulfan, endosulfan sulfate, endosulfan diol, endosulfan lactone, and potential conjugates of specific metabolites (Table 17 and Figure 6). In all required poultry tissues, individual unidentified residues each represented less than 10% TRR and/or less than 0.05 ppm.

13. All of the residues identified contained intact chlorinated rings, including all labeled carbons, even after treatments to cleave conjugates. Identified metabolites represented 50% TRR in muscle and higher portions in other tissues. This information indicates the stability of labeled rings. With this consideration and Conclusion 12, the nature of the residue in poultry is adequately understood.

Recommendations

In accordance with Conclusions 1 through 7, the metabolic pathway of endosulfan is similar in three diverse crops, and Guideline 860.1300 is satisfied for crops. The data submitted, including data on leaves, will be presented to the HED Metabolism Committee for a determination of the residues to be regulated in plants.

In accordance with Conclusions 8 through 13, Guideline 860.1300 is satisfied for livestock. The data submitted will be presented to the HED Metabolism Committee for a determination of the residues to be regulated in livestock.

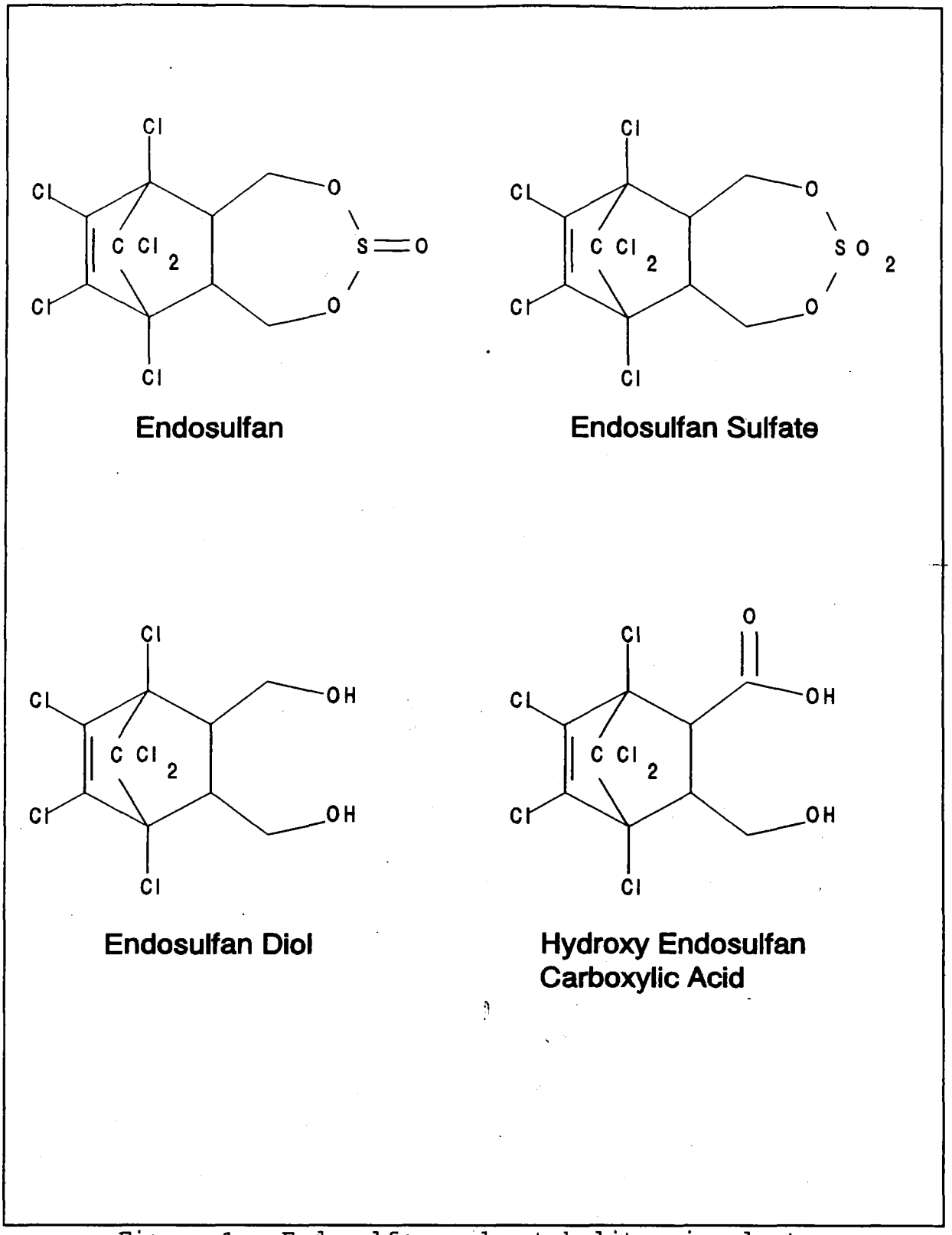

Figure 1. Endosulfan and metabolites in plants.

DETAILED CONSIDERATIONS

Background

The Update to the Residue Chemistry Chapter (8/9/90) required the following data on plant and animal metabolism:

Data depicting the uptake, distribution, and metabolism of uniformly ring-labeled [^{14}C]endosulfan in three dissimilar food crops (e.g., alfalfa, apples, and beans) at levels sufficient to make residue identification and quantification possible are required. If metabolism is not similar in the three crops additional studies using other crops may be required. A completely characterized test substance representative of technical endosulfan (including impurities, if appropriate) used in commercial formulations must be applied under conditions representing normal cropping practices to the test crops at exaggerated rates (2-5x) to permit characterization of ^{14}C residues. Confirmation of the identities of residues using a suitable method such as MS or HPLC is also required. In addition, representative samples from these tests must be analyzed using accepted enforcement methods to ascertain that these methods will recover and quantify all metabolites of concern.

Metabolism studies must be conducted utilizing ruminants and poultry. Animals must be dosed orally for a minimum of 3 days with uniformly ring-labeled [^{14}C]endosulfan in the diet at a level sufficient to make residue identification and quantification possible. Milk and eggs must be collected twice daily during the dosing period. Animals must be sacrificed within 24 hours of the final dose. The distribution and identity of residues must be determined in milk, eggs, liver, ruminant kidney, muscle, and fat. Representative samples from the required metabolism studies must also be analyzed using a suitable confirmatory method such as MS or HPLC. In addition, representative samples from [^{14}C]endosulfan metabolism studies must also be analyzed by the residue analytical methods developed for data collection and tolerance enforcement to ascertain that the methods are capable of adequately recovering and quantifying all residues of concern.

Registrant AgrEvo, representing the Endosulfan Task Force, subsequently met with EPA personnel including representatives of CBRS, to discuss endosulfan metabolism studies (Memo, 11/29/94, F.B. Suhre). The Registrant advised that metabolism studies had been initiated with endosulfan labeled in the 5a and 9a positions (see Figure 3). CBRS advised that the evidence provided by the Registrant indicated that this label was stable in plant metabolism studies; evidence to support stability of the label

should be provided for rotational crop and livestock metabolism studies.

It was subsequently reported that among other crops, alfalfa was not being supported for reregistration (Memo, 6/27/95, J.M. Wintersteen). Therefore, contrary to the suggestion in the Update, this crop would not be appropriate for a plant metabolism study.

LETTUCE

Field Procedures

The following study was submitted:

Metabolism of [^{14}C]-Endosulfan in Lettuce, AgrEvo USA Company, Report A55811, August 5, 1996 (MRID 44082702).

The performing laboratory for both the agricultural and analytical portions of the study was AgrEvo Research Center, Environmental Chemistry Department, Pikeville NC. Lettuce was grown in sandy loam soil in a steel tank. The test area was enclosed by a wooden cage, surrounded by wire mesh to keep out wildlife, and covered with transparent sheeting to keep out rain.

The test substance was a 3 lb/gal EC formulation, containing endosulfan labeled with ^{14}C at the 6, 7, 8, 9, and 10 ring positions; the ratio of alpha to beta isomers was approximately 2:1 (Figure 2 shows the labels on the alpha isomer; the same atoms were also labeled on beta isomer in the test substance). The label was mixed with unlabeled endosulfan to a specific activity of 15 μCi/mg; radiochemical purity was 97%.

Lettuce plants (leafy variety Black Seeded Simpson) were transplanted from a greenhouse to the test area on September 21, 1995. Test substance was applied by hand sprayer at 21 days (October 19) and 14 days before final harvest, at 1 lb ai/A for each application.

Lettuce plant samples were collected by hand at 0, 7, 14, and 21 days after the first treatment; final harvest was November 9, 1995. Duplicate samples were extracted on the day of each harvest; all other samples were placed into frozen storage and maintained at -15°C or colder when not being analyzed.

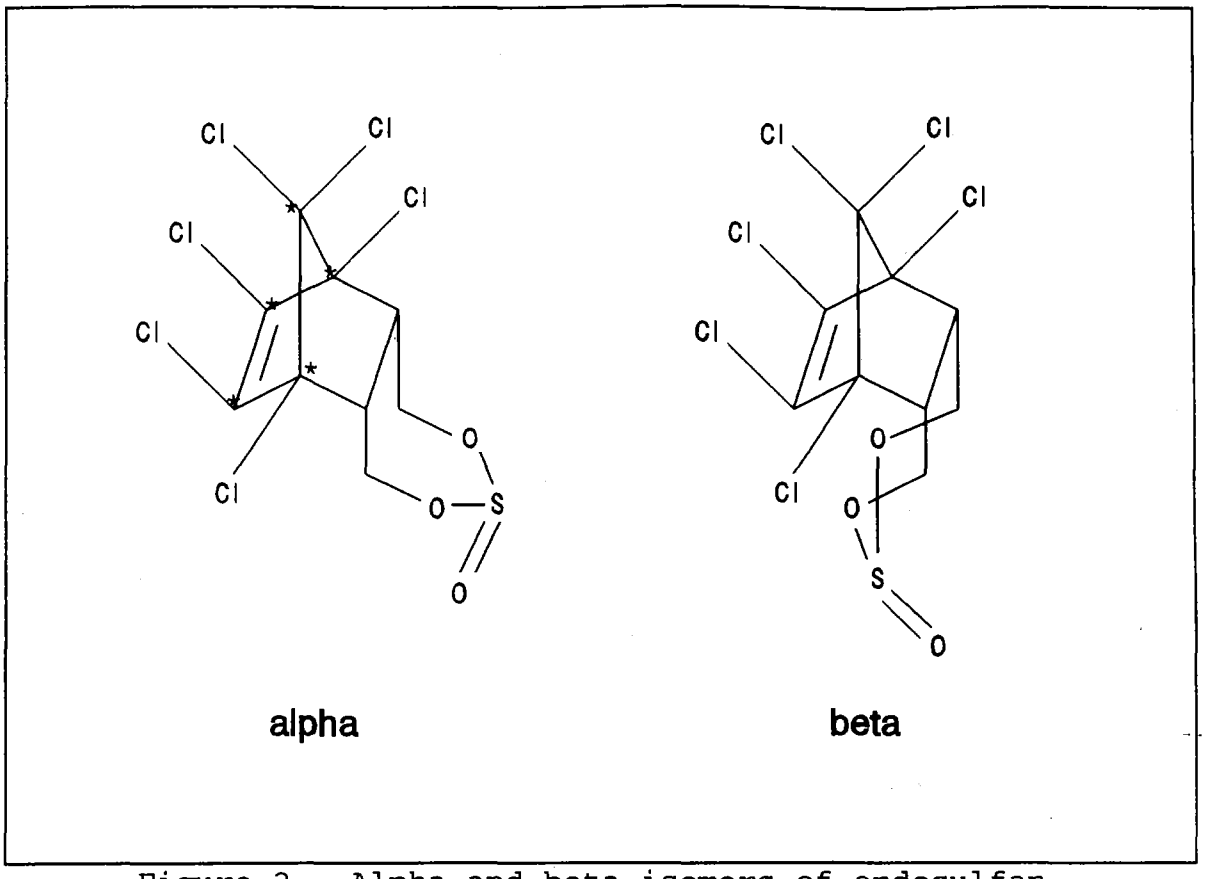

Figure 2. Alpha and beta isomers of endosulfan (* on alpha = labeled carbons 6,7,8,9,10).

Laboratory Analysis

Harvested lettuce samples were washed with acetonitrile. Washed samples were extracted by blending with acetonitrile, and the extract was filtered under vacuum. With the final harvest (day 21) samples, the filtered solid was extracted overnight by Soxhlet treatment in water. Total radioactive residues (TRR) of liquid samples were determined by adding aliquots to scintillation cocktail and then determining radioactivity by liquid scintillation counting (LSC); TRR of filtered solids were determined by combustion of aliquots in a sample oxidizer and LSC. Results of extractions are summarized in Table 1:

Table 1. Extraction and distribution of TRR in lettuce samples.

Fraction	% TRR (ppm) by days after first application:			
	0 days	7 days	14 days	21 days
Acetonitrile Wash	87.3 (290.2)	21.2 (7.0)	27.4 (25.4)	22.6 (15.3)
Acetonitrile Extract	11.5 (42.6)	67.2 (20.6)	61.1 (56.0)	66.4 (44.4)
Soxhlet				5.1 (3.4)
Unextracted	1.2 (3.6)	11.6 (3.5)	11.5 (10.8)	5.9 (4.1)
TRR, ppm	(336.4)	(31.1)	(92.1)	(67.2)

Table note: TRR values are averages of two determination. Only 21 day samples were treated by Soxhlet.

Residue Analysis

Extracts were analyzed by HPLC using a Zorbax ODS column, eluted with acetonitrile:water:glacial acetic acid (60:40:1), isocratic. Assignment based on HPLC was confirmed by thin layer chromatography (TLC) using silica gel plates, developed with hexane:acetone (10:3) in one dimension. Residues were assigned based on comparison with mobilities of standards.

Based on the combined analytical data, residues identified in lettuce were both parent isomers, the sulfate, and small amounts of the diol. The Registrant noted that the ratio of alpha:beta endosulfan in plant samples decreased with time; this was attributed to greater volatization of the alpha isomer. Unknown residues each represented less than 10% TRR. Results are summarized in Table 2:

Table 2. Residues identified in lettuce samples.

Residue	% TRR (ppm) by days after first application:			
	0 days	7 days	14 days	21 days
Alpha Endosulfan	63.3 (212.9)	28.3 (8.80)	22.9 (21.1)	21.0 (14.1)
Beta Endosulfan	31.4 (105.6)	47.0 (14.6)	49.5 (45.6)	47.6 (32.0)
Endosulfan Sulfate	0.5 (1.68)	9.3 (2.89)	11.6 (10.7)	15.4 (10.3)
Endosulfan Diol	1.8 (6.06)	0.8 (0.25)	1.0 (0.92)	2.9 (1.95)
Total Identified	97.0	85.4	85.0	86.9

Table notes: See Figures 1 and 2 for structures.

CBRS Comments

Extracts were stored frozen prior to analysis. The Registrant noted that analysis for this metabolism study was completed within 3 months of the final harvest. Storage stability data therefore are not required. The considerations above lead to the following comment:

Conclusion 1: In mature lettuce, 87% of total residues were identified. The large majority of residues consisted of the alpha and beta isomers of endosulfan; smaller amounts of endosulfan sulfate, and small amounts of endosulfan diol were also present. Individual unidentified residues each represented less than 10% TRR. (See Figures 1 and 2 and Table 2).

The Update to the Residue Chemistry Chapter (8/9/90) described use directions on lettuce. The least restrictive directions were three foliar applications to head lettuce after thinning, maximum rate for each application 1.1 lb ai/A; or two foliar applications per season to leaf lettuce, maximum rate for each application 1 lb ai/A. A 14 day PHI is established. The wrapper leaf is to be removed from head lettuce.

According to Branch Cultural Practice files, thinning of lettuce occurs 2-4 weeks after planting (seed), and a lettuce head may be fully developed in about 60 days. The application parameters for this study are therefore consistent with use directions for leaf lettuce. An additional application at the same rate would be

allowed for head lettuce. However, a high proportion of the residue was identified in this study, and the most extensively metabolized residue, the diol, represented only a small percent of TRR. An additional application would not be expected to produce dramatically different results for metabolism in lettuce. The considerations above lead to the following comment:

Conclusion 2: Conditions for this study, two applications at 21 and 14 days pre-harvest, each at 1 lb ai/A, represented approximately the maximum rate for leaf lettuce. The nature of the residue in lettuce is adequately understood.

CUCUMBER

Field Procedures

The following study was submitted:

Endosulfan Metabolism in Cucumber (Cucumis Sativus) Following Three Treatments with the ^{14}C-Labeled Test Substance at 7-Day Intervals and a Nominal Rate of 530 g ai/ha Each, AgrEvo USA Company, Report A56011, November 27, 1995 (MRID 44099101).

The performing laboratory for both the field and analytical portions of the study was Hoechst Schering AgrEvo GmbH, Frankfurt, Germany. Cucumbers were transplanted to a plant box containing sandy loam soil on June 28, 1993. The plant box was placed in an outdoor vegetation room, surrounded by wire mesh fencing to keep out birds, and covered by a glass roof to keep out rain.

The test substance was an EC 35 formulation, containing endosulfan labeled with ^{14}C at the 5a and 9a ring positions; the ratio of alpha to beta isomers was approximately 2:1 (Figure 3 shows the labels on the alpha isomer; the same atoms were also labeled on beta isomer in the test substance). Specific activity was 70,500 dpm/μg, and radiochemical purity was 97%. Test substance was applied by hand sprayer at 0.53 kg ai/ha (0.47 lb ai/A), in each of three applications, which were 28, 21, and 14 days before harvest. The present submission noted that in the United States, 6 applications per season were permitted with a maximum seasonal rate of 3.3 kg ai/ha (2.9 lb ai/A).

Leaves and fruit were collected after the last application by cutting them off with scissors. A few leaves and fruit samples were collected at 0, 3, and 7 days, and remaining plants were harvested 14 days after the last application. Untreated control plants were collected at the same times. First aliquots of samples were analyzed within three weeks of sampling. Samples were placed into frozen storage and maintained at -20°C or colder when not being analyzed.

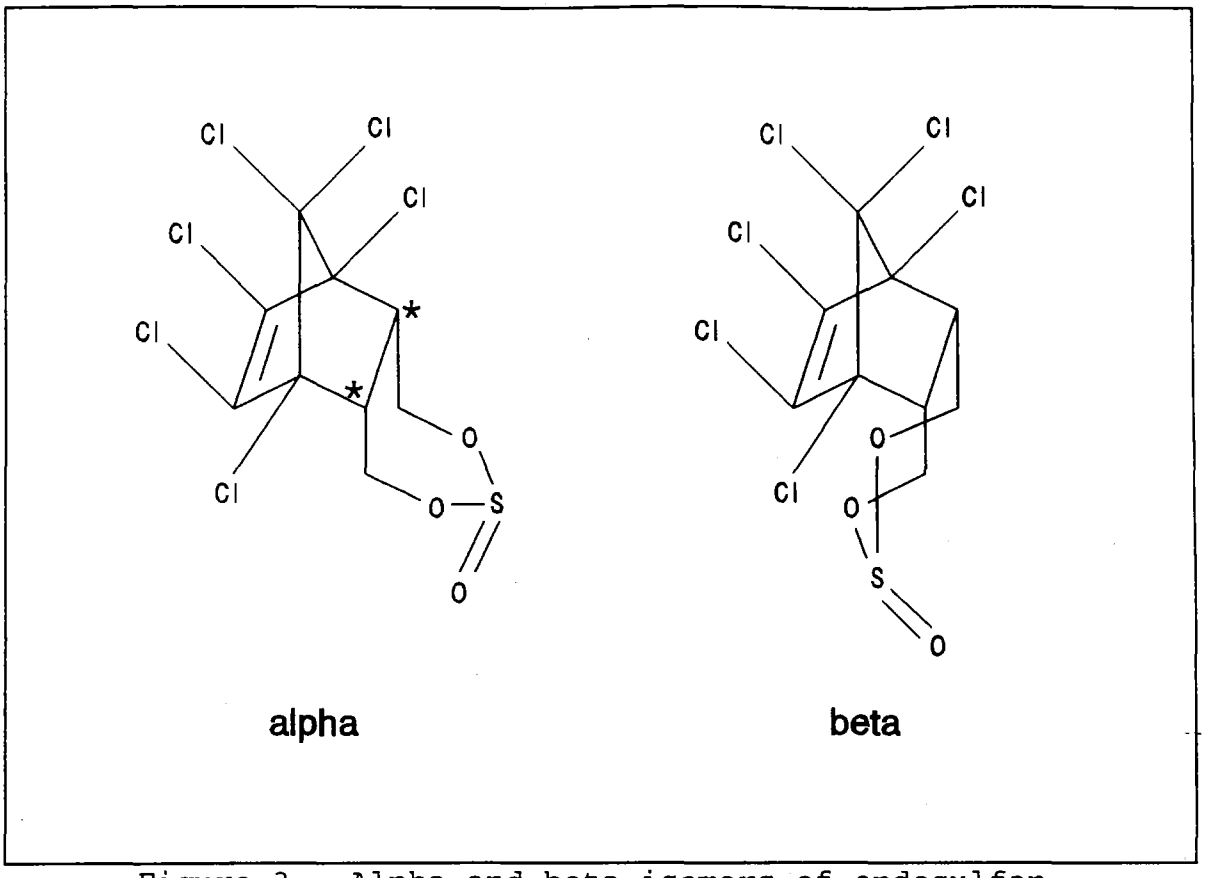

Figure 3. Alpha and beta isomers of endosulfan (* on alpha = labeled carbons 5a and 9a).

Laboratory Analysis

Leaf samples were homogenized with dry ice in a blender. Fruit was chopped with a knife and homogenized with water in a blender. Leaf pieces and aliquots of fruit pulp were analyzed for total radioactive residues (TRR) by combustion in a sample oxidizer and liquid scintillation counting (LSC). TRR in leaves varied with time after last application; the Registrant attributed this to non-homogenous application of chemical due to many overlapping leaves. Results are summarized in Table 3:

Table 3. Total radioactive residues (TRR) in cucumber samples.

Days after last application	TRR, ppm, in:	
	Fruit	Leaves
0	0.24	185
3	0.26	87
7	0.25	18
14	0.18	52

Cucumber fruit and leaves were extracted in a similar manner, although the procedure for leaves was somewhat more extensive. The basic extraction protocol for each is outlined in Figure 4. Fruit was extracted three times with water:acetone (1X 1:40, 2X 1:9), the mixture was centrifuged, and supernatants were combined. Acetone was evaporated with nitrogen, and the resulting solution was partitioned with dichloromethane into organic and aqueous fractions. For fruit harvested 14 days after the last application, the aqueous fraction was treated by two different procedures. In one procedure, the aqueous phase was hydrolyzed by reflux for 3 h in 10 M HCl, followed by neutralization with NaOH, and partition with dichloromethane; neutralization produced a salt precipitate. In the second procedure, the aqueous fraction was purified by solid phase extraction with an RP18 column, which produced a percolate fraction and an eluate fraction. The percolate was then taken through HCl hydrolysis and dichloromethane partitioning as described above.

Leaf samples were taken through a similar protocol as fruit, except that the protocol was extended for leaves collected 14 days after the last application. For these samples, aqueous fraction was treated by one of three procedures: hydrolysis, as for fruit; solid phase extraction, but without the percolate fraction; or semi-preparative thin layer chromatography, which produced three main peaks, each of which was scrapped off and extracted with acetonitrile. Table 4 summarizes the extraction of 14 day fruit, and Table 5 summarizes extraction of 14 day leaves:

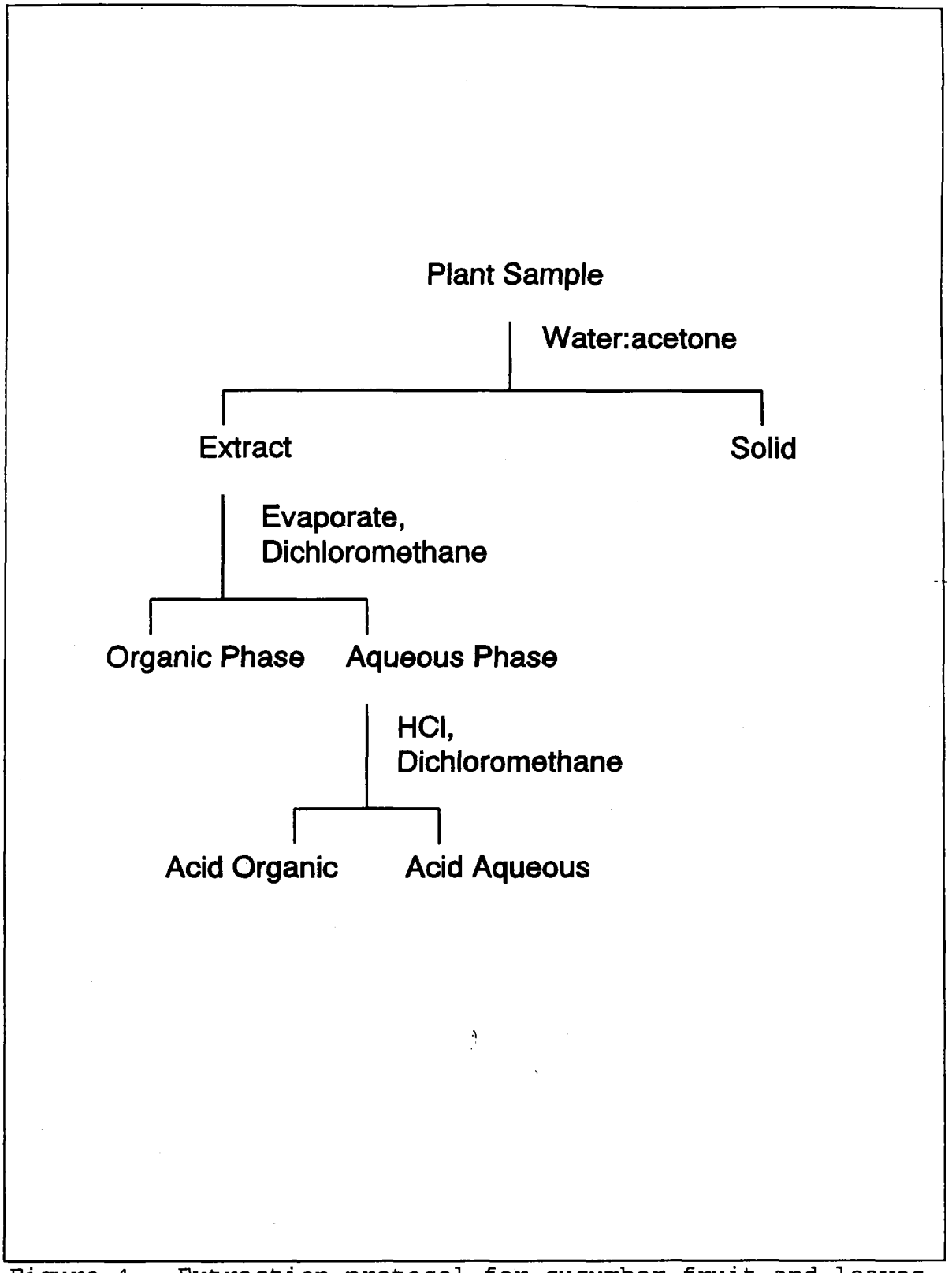

Figure 4. Extraction protocol for cucumber fruit and leaves.

Table 4. Extraction and distribution of TRR in cucumber fruit.

Fraction	% TRR (ppm) in fraction from:		
	Extraction A	Extraction B	Average
TRR (ppm)	100 (0.198)	100 (0.077)	100 (0.138)
Organic	51.0 (0.101)	63.4 (0.049)	57.2 (0.044)
Aqueous [further extracted]	[34.4] (0.068)	[25.0] (0.019)	[29.7] (0.044)
Solid Phase Eluate	16.5 (0.033)		
Acid Organic	0.1 (<0.001)	2.7 (0.002)	
Acid Aqueous	5.2 (0.010)	6.0 (0.005)	
Acid Precipitate	3.0 (0.006)	9.9 (0.008)	
Unextracted Solid	12.6 (0.025)	10.1 (0.008)	11.4 (0.016)

Table notes: Data are from fruit harvested 14 days after the last application. See Figure 4 for the basic protocol; additional details are described in the text. In Extraction A, the solid phase percolate was hydrolyzed in acid; in Extraction B, the aqueous fraction was hydrolyzed directly.

Table 5. Extraction and distribution of TRR in cucumber leaves

Fraction	% TRR in fraction from:		
	Extraction A	Extraction B	Extraction B, TLC
Organic	45.0	42.7	Same as Extraction B
Unextracted Solid	22.1	16.7	
Aqueous [further extracted]	[29.8]	[33.5]	
Solid Phase Eluate	23.6		
Acid Organic		13.9	
Acid Aqueous		9.3	
TLC, Peak 1			22.7
TLC, Peak 2			5.2
TLC, Peak 3			5.6

Table notes: Data are from leaves harvested 14 days after the last application. See Figure 4 for the basic protocol; additional details are described in the text. In Extraction A, the aqueous phase was purified by solid phase extraction. In Extraction B, aliquots of the aqueous phase were hydrolyzed in acid, or purified by TLC. TRR was 52 ppm (see Table 3).

Residue Analysis

To identify residues, extracts were analyzed by reverse phase HPLC, by thin layer chromatography (TLC), by gas chromatography-mass spectroscopy (GC-MS), or by HPLC-MS. HPLC was performed with an LiChrosorb RP-18 column, eluted with gradients in 0.1 M ammonium acetate as Solvent A and acetonitrile as Solvent B; with some analyses, when the gradient reached 100% B, remaining metabolites were eluted with 100% dioxane. Assignment based on HPLC was confirmed by thin layer chromatography (TLC) using silica gel plates, developed with hexane:acetone (10:1) in one dimension. For semi-preparative TLC, aqueous extracts were spotted on siliga gel plates, developed with ethyl acetate:2-propanol:water (65:23:12) in one dimension. Residues were assigned based on comparison with mobilities of standards.

The organic extract from cucumber leaves was analyzed by GC-MS using a FISONS Model 8065 apparatus with a DB-1 column; the mass spectrometer was VG/FISONS TRIO 2000; ionization mode was electron impact. The aqueous extract from cucumber leaves was

analyzed by HPLC using an Alltima (Alltech) column, eluted with gradients in the same Solvents A and B described above; the mass spectrometer was Finnigan TSQ 700, operated with electrospray ionization and atmospheric pressure chemical ionization. Mass spectra were compared with those of standard compounds.

When organic extracts from fruit were analyzed by HPLC, five peaks were observed; the three strongest were assigned to alpha and beta endosulfan, and the sulfate (Figure 1). These assignments were confirmed by TLC analysis. When the solid phase eluate was analyzed by HPLC, approximately 10 peaks, each representing less than 10% TRR, were observed. Fractions generated by acid hydrolysis of the aqueous extract, and unextracted solids, were not further analyzed because of low levels of radioactivity (Table 4).

Because TRR in leaves was considerably greater than in fruit, additional work was conducted with leaf extracts. When organic extracts from leaves were analyzed by HPLC, three strong peaks, representing alpha and beta endosulfan and the sulfate, were observed. Assignments were confirmed by TLC, and identity of the sulfate was confirmed by GC-MS. Analysis of the solid phase eluate from the aqueous fraction gave results similar to those in fruit: approximately 10 peaks, each representing less than 5% TRR, were observed. When the aqueous extract was hydrolyzed and partitioned (Table 5, Extraction B), HPLC analysis of the acid organic fraction identified the endosulfan diol (Figure 1), representing 5.7% TRR. This suggested that the diol had been cleaved from a conjugate.

The aqueous fraction from leaves was also purified by semi-preparative TLC, producing three major peaks. These peaks were analyzed by HPLC-MS and determined to consist of multiple sugar conjugates of hydroxy endosulfan carboxylic acid; multiple sugar conjugates of endosulfan diol plus a small amount (1.3% TRR) of the free hydroxy carboxylic acid; and a mono-hexose conjugate of the diol, respectively (see Figure 1 for structures of the free compounds). Unextracted solids from leaves were not further analyzed because these fractions were barely 10% TRR in fruit samples (Table 4). Identification of residues in cucumber fruit and leaves is summarized in Table 6:

Table 6. Residues identified in cucumber samples.

Residue	% TRR (ppm) in mature:	
	Fruit	Leaves
Alpha Endosulfan	14.5 (0.026)	7.1 (3.7)
Beta Endosulfan	14.6 (0.026)	11.0 (5.7)
Endosulfan Sulfate	21.4 (0.038)	17.7 (9.2)
Endosulfan Diol, sugar conjugates		9.5 (4.9)
Hydroxy Endosulfan Carboxylic Acid, free and sugar conjugates		24.0 (12.5)
Total Identified	50.5 (0.09)	69.3 (36.0)

Table notes: Data are summarized for samples harvested 14 days after the last application. Blank spaces indicate residues not identified. See Figures 1 and 2 for structures.

CBRS Comments

No storage stability data were submitted in support of the study. However, final harvest was on August 27, 1993, and laboratory analysis was completed March 1, 1994. Laboratory analysis was therefore substantially complete within 6 months of harvest. The considerations above lead to the following comment:

Conclusion 3: In mature cucumber fruit, 50% of total residues were identified as alpha and beta endosulfan and endosulfan sulfate. In mature cucumber leaves, 69% of total residues were identified. Residues identified were the same as those in fruit, plus sugar conjugates of endosulfan diol and hydroxy endosulfan carboxylic acid; conjugates represented more than 30% of TRR in leaves. (See Figures 1 and 2 and Table 6).

The test substance for this study was formulated to correspond to Thiodan EC 35. A recent copy of this label is included in Crop Protection Chemicals Reference, 1994, Chemical and Pharmaceutical Publishing Corporation, New York. For cucumbers, the maximum number of applications is 6 per year, applied weekly; maximum rates are 1 lb ai/A for each application, with an annual maximum of 3 lb ai/A; and a PHI of 2 days is established. With the maximum number of applications, therefore, cucumbers could be harvested 37 days after the first application.

For this metabolism study, harvest was 14 days after the last application and 28 days after the first application, and the combined rate from three applications was 1.4 lb ai/A. The applications during this study therefore represented half the maximum number of applications, and approximately half the maximum annual rate. This was not consistent with the requirements of the Update, which specified normal cropping conditions and exaggerated rates (see Background section above). If these requirements had been followed, then residues in cucumbers would have been higher in magnitude, and metabolism could have been more extensive. We note in addition that the established tolerance for cucumbers is 2 ppm for combined residues of parent and the sulfate only (40 CFR 180.182), much higher than residues from the submitted study.

Further analysis of aqueous fractions from fruit was limited in this study because of low levels of radioactivity. However, the proportion of TRR represented by aqueous fractions was similar in fruit and leaves (see Tables 4 and 5), and the additional work to identify polar residues in leaves can be translated to fruit. These considerations lead to the following comment:

Conclusion 4: Applications during this study represented half the maximum annual number, and approximately half the maximum annual rate. These conditions were not consistent with the requirements of the Update to the Residue Chemistry Chapter (8/9/90), which specified normal cropping conditions and exaggerated rates. However, the additional work on polar residues in leaves can be translated to fruit. With the data from leaves, the nature of the residue in cucumber is adequately understood.

APPLES

Field Procedures

The following study was submitted:

Endosulfan: Metabolism in Apples (Malus Sylvestris var. Domesticia) Following Single Treatment of a Young Tree with ^{14}C-Labelled Test Substance, AgrEvo USA Company, Report A53662, January 19, 1995 (MRID 44082701).

The performing laboratory for both the field and analytical portions of the study was Hoechst Schering AgrEvo GmbH, Frankfurt, Germany. Application was to a 6 year old apple tree, nearly 2 m tall, growing in a cylindrical pot; a similar tree was used as a control. The trees had been grown outdoors; before the study, they were transferred to a roofed-over outdoor vegetation hall, surrounded by wire mesh fencing to keep out birds.

The test substance was an EC 35 formulation, containing endosulfan labeled with ^{14}C at the 5a and 9a ring positions; the ratio of alpha to beta isomers was approximately 2:1 (Figure 3 shows the labels on the alpha isomer; the same atoms were also labeled on beta isomer in the test substance). Specific activity was 70,500 dpm/μg, and radiochemical purity was 97%. Test substance was applied by ground sprayer at 1.5 kg ai/ha (1.34 lb ai/A). Blank formulation with no endosulfan was applied to the control tree.

At the time of application on 8/26/93, the test tree contained 13 apples. Apples and leaves were harvested by hand at 0, 7, 14, and 21 days after application. Samples were placed into frozen storage and maintained at -20°C or colder when not being analyzed.

The present submission noted that in the United States, 3 applications per season were permitted with a maximum seasonal rate of 3.3 kg ai/ha (2.9 lb ai/A).

CBRS Comment, Field Procedures

The Update to the Residue Chemistry Chapter (8/9/90) described use directions on apples. The least restrictive directions were three foliar applications during the fruiting season at 2.5-4 lb ai/A maximum seasonal rate, with a 21 day PHI.

The test substance for this study was formulated to correspond to Thiodan EC 35, as with the study in cucumber. The label for this formulation allows first application before petal fall, which would be several months before harvest. The considerations above lead to the following comment:

Conclusion 5: Application for this study represented approximately the maximum rate for the single application closest to harvest (21 day PHI). Use directions allow additional applications much earlier in the season, which would be expected to cause more extensive metabolism by harvest.

Laboratory Analysis

Harvested apples were rinsed with acetone, then homogenized in a blender. Leaf samples were homogenized with dry ice in a blender. Aliquots were taken for combustion, other aliquouts were extracted, and remaining samples were stored in a freezer until further analysis. Total radioactive residues (TRR) of homogenized samples were determined by combustion of aliquots in a sample oxidizer and liquid scintillation counting (LSC); aliquots of acetone rinses were added to scintillation cocktail and counted by LSC. TRR in apples showed non-linear variations with time; the Registrant attributed this to non-homogenous

application of chemical to the test tree. Results are summarized in Table 7:

Table 7. Total radioactive residues (TRR) in apple samples.

Days post-treatment	TRR, ppm, in:	
	Apples	Leaves
0	0.44	80.6
7	1.37	35.0
14	0.74	33.3
21	0.98	24.5

Table note: TRR includes data on acetone rinses for apples.

Homogenized apple samples were extracted three times with acetone:water (9:1). Extracts were combined, concentrated by rotary evaporation, and then partitioned with dichloromethane into organic and aqueous phases. Leaves were extracted in the same manner as apples, except leaves were not first rinsed in acetone. Table 8 summarizes the results of extraction:

Table 8. Extraction and distribution of TRR in apple samples.

Fraction	% TRR (ppm) by days after treatment:			
	0 days	7 days	14 days	21 days
Apples:				
Acetone Rinse	47.1 (0.207)	36.4 (0.499)	10.3 (0.076)	24.0 (0.235)
Organic	50.2 (0.221)	57.8 (0.792)	82.3 (0.609)	71.8 (0.704)
Aqueous	1.1 (0.005)	3.5 (0.048)	5.0 (0.037)	3.3 (0.032)
Unextracted	1.1 (0.005)	1.8 (0.024)	2.3 (0.017)	1.1 (0.011)
TRR, ppm	(0.44)	(1.37)	(0.74)	(0.98)
21-day leaves:				
Organic				74.7 (18.3)
Aqueous				13.1 (3.2)
Unextracted				9.8 (2.4)
TRR, ppm				(24.5)

Table notes: Extraction is described in the text. Acetone rinse was performed on apples only. With leaves, only 21 day samples were extracted.

Residue Analysis

Extracts were analyzed by reverse phase HPLC using a LiChrosorb RP-18 column, eluted with gradients in acetonitrile as Solvent A, and 0.1 M ammonium acetate as Solvent B. Assignment based on HPLC was confirmed by thin layer chromatography (TLC) using silica gel plates, developed with hexane:acetone (10:1) in one dimension. Residues were assigned based on comparison with mobilities of standards. With apple fruits, the acetone rinse and organic extracts were analyzed by HPLC; assignment of residues from 21 day extracts was confirmed by TLC analysis. With leaves, aqueous and organic extracts were analyzed by HPLC.

Residues in leaf extracts were also isolated by preparative TLC using silica gel plates, developed prior to extract application with methanol and then with hexane:dichloromethane (10:3). After

application of extract, plates were developed in one dimension with hexane:dichloromethane. Two strong peaks were observed; each was scraped off, eluted with acetonitrile, and analyzed by gas chromatography-mass spectroscopy (GC-MS). Gas chromatography apparatus was FISONS Model 8065; the mass spectrometer was VG/FISONS TRIO 2000; ionization mode was electron impact. Mass spectra were compared with those of standard compounds alpha endosulfan, beta endosulfan, and endosulfan sulfate.

Based on the combined analytical data, residues identified in apples were both parent isomers and the sulfate. Residues identified in leaves were these same compounds, plus small amounts of the diol. The Registrant noted that the ratio of alpha:beta endosulfan in plant samples was lower than in the test substance applied; this was attributed to greater volatization of the alpha isomer. Results are summarized in Table 9:

Table 9. Residues identified in apple samples.

Residue	% TRR (ppm) by days after treatment:			
	0 days	7 days	14 days	21 days
Apples:				
Alpha Endosulfan	54.3 (0.239)	49.7 (0.678)	47.9 (0.354)	50.7 (0.499)
Beta Endosulfan	43.1 (0.189)	44.0 (0.601)	43.4 (0.320)	43.1 (0.425)
Endosulfan Sulfate		0.9 (0.012)	1.5 (0.011)	1.5 (0.015)
Total Identified	97.4 (0.428)	94.6 (1.291)	92.8 (0.685)	95.3 (0.939)
21-day leaves:				
Alpha Endosulfan				7.6 (1.85)
Beta Endosulfan				28.3 (6.93)
Endosulfan Sulfate				49.6 (12.12)
Endosulfan Diol				0.9 (0.21)
Total Identified				86.4 (21.11)

Table notes: See Figures 1 and 2 for structures.

CBRS Comments

No storage stability data were submitted in support of the study. However, mass spectra included in the present submission were dated December 22, 1993. The first apple samples were harvested on August 26, 1993. Therefore, samples were stored for fewer than 6 months prior to analysis. The considerations above lead to the following comment:

Conclusion 6: In apples harvested 21 days after application, 95% of total residues were identified. The large majority of residues in fruit consisted of the alpha and beta isomers of endosulfan; small amounts of the sulfate were also present. In 21 day leaves, 86% of total residues were identified. The sulfate represented nearly half the residues in leaves; alpha and beta endosulfan were also identified, along with small amounts of the diol. In fruit and leaves, individual unidentified residues each represented less than 10% TRR. (See Figures 1 and 2 and Table 9).

In the present submission, the sulfate represented less than 2% TRR in mature fruit, and the diol less than 1% TRR in mature leaves (Table 9). The submitted results indicate if metabolism in apples had been more extensive by an order of magnitude, the sulfate in fruit and the diol in leaves would each barely represent 10% TRR. Thus, metabolism in apples would have been no more extensive than the situation observed with cucumber. These considerations lead to the following comments:

Conclusion 7: As Conclusion 5 indicates, a metabolism study with applications earlier in the season, consistent with registered use, would have been preferable for apples. However, if metabolism in apples were considerably more extensive than the submitted results indicate, the array of metabolites produced would be expected to be no greater than that observed with cucumber (Conclusion 3). With the combined data on apple fruit and leaves, the nature of the residue in apples is adequately understood.

Recommendation: In accordance with Conclusions 1 through 7, the metabolic pathway of endosulfan is similar in three diverse crops, and Guideline 860.1300 is satisfied for crops. The data submitted, including data on leaves, will be presented to the HED Metabolism Committee for a determination of the residues to be regulated in plants.

COW

Livestock Procedures

The following study was submitted:

Endosulfan: Distribution, Elimination and the Nature of the Metabolite Residues in the Milk and Edible Tissues of a Lactating Cow, AgrEvo USA Company, Report A57041, May 21, 1996 (MRID 44082703).

The performing laboratory for animal dosing and laboratory analysis was AgrEvo UK Limited, Toxicokinetics, Essex, England. Test substance was [^{14}C]endosulfan, labeled at the 6, 7, 8, 9, and 10 ring positions; the ratio of alpha to beta isomers was approximately 70:30 (Figure 2 above shows the labels on the alpha isomer; the same atoms were also labeled on beta isomer in the test substance). Specific activity was 27,800 dpm/μg, and radiochemical purity was 98%.

Test substances were placed in gelatin capsules and a cow was dosed once daily at approximately 22 ppm in the diet, for five consecutive days. The cow was milked twice daily, in the morning and afternoon. The cow was sacrificed approximately 22 h after receiving the last dose. Liver, kidneys, heart, muscle (loin and hindquarter), and fat (renal, subcutaneous, and omental) were removed and stored at approximately -20°C until analysis. Urine and feces were also collected to account for the distribution of the administered dose.

Laboratory Analysis

To determine total radioactive residues (TRR) in tissues, samples were minced and aliquots were placed overnight in solubilizing solution, a mixture of aqueous sodium hydroxide, Triton detergent, and methanol. The mixture was neutralized with acetic acid, then added to scintillation cocktail, and radioactivity was determined by liquid scintillation counting (LSC). Milk samples were mixed with cocktail and counted directly. Total residues are summarized in Table 10. Milk residues reached a maximum of 0.171 ppm after the fourth daily dose. The performing laboratory reported that radioactivity in urine and feces accounted for 44% or more of the administered dose.

Table 10. Total radioactive residues in cow.

Tissue	TRR, ppm
Liver	3.572
Kidney	0.785
Heart	0.161
Muscle, hindquarter	0.031
Muscle, loin	0.052
Milk	0.171
Omental fat	1.278
Renal fat	0.840
Subcutaneous fat	0.305

The extraction protocol was most extensive for liver and kidney, and is outlined in Figure 5. Tissue samples were homogenized in hexane, and extracted by stirring overnight. The homogenized sample was centrifuged and the supernatant was removed. This hexane extract was applied to a silica Mega Bond Elut column, the flow-through was saved as the hexane fraction, and the column was then eluted with acetonitrile. The pellet from hexane extraction was resuspended and extracted in acetonitrile. After centrifugation and removal of supernatant, the remaining pellet was extracted in acetonitrile:water (70:30). The extracts from these steps were added to the eluant from the silica column to form a combined acetonitrile extract.

The pellet from acetonitrile:water extraction was resuspended and extracted in methanol. After centrifugation and removal of supernatant, the remaining pellet was extracted in water. The pellet from water extraction was treated sequentially with enzymes. Successive treatments were with collagenase, pancreatin (containing amylase, trypsin, lipase, ribonuclease, and protease), gamma-glutamyl transpeptidase, carboxypeptidase, a second treatment with pancreatin, then proteinase K. The pellet remaining from all enzyme treatments was refluxed in 1 N HCl. The pellet remaining from HCl reflux was then treated with 1 N NaOH.

In a separate analysis, the water fraction and the combined enzyme fractions were pooled. An aliquot was refluxed in 6 N HCl, then extracted into ethyl acetate.

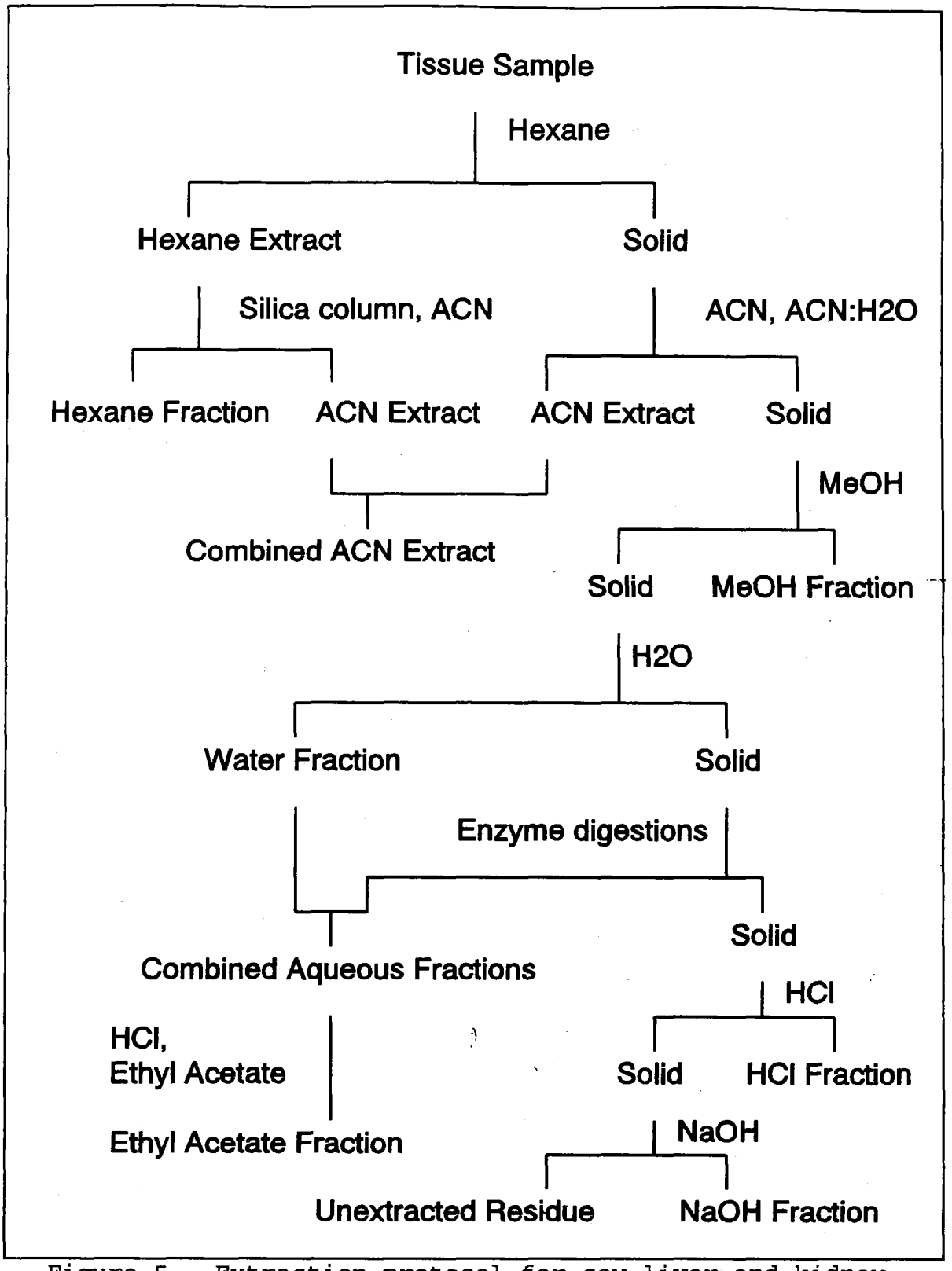

Figure 5. Extraction protocol for cow liver and kidney.

Other tissues were extracted using similar but less extensive protocols. Heart was extracted through the combined enzyme digestions, but no further. Loin muscle was extracted through the water fraction, and the resulting solid was treated with collagenase, then pancreatin. Milk was extracted through the combined acetonitrile extract.

Extraction of fat samples was slightly different. Subcutaneous fat was extracted through the combined acetonitrile extract, but the acetonitrile:water extract was saved as a separate fraction, not combined with acetonitrile extracts. Omental and renal fat were extracted in a similar manner as in Figure 5 to produce a combined acetonitrile extract, except that an acetonitrile:water extraction was not performed. In addition, the hexane extract was centrifuged before addition to the silica column, the resulting pellet was resuspended in hexane, and most of the radioactivity was partitioned to an additional acetonitrile fraction. Tables 11 and 12 summarize the distribution of radioactive residues in cow tissues:

Table 11. Distribution of radioactive residues in cow liver, kidney, muscle, and heart.

Fraction	Recoveries in % TRR (ppm) from tissue [TRR in ppm]:			
	Liver [3.572]	Kidney [0.785]	Loin muscle [0.052]	Heart [0.161]
Hexane	0.7 (0.024)	<1.0 (<0.01)	<1.0 (<0.001)	<1.0 (<0.002)
Combined acetonitrile	30.6 (1.093)	28.5 (0.224)	65.9 (0.034)	49.2 (0.079)
Methanol	1.7 (0.062)	3.9 (0.031)	0.4 (<0.001)	1.3 (0.002)
Water	5.6 (0.201)	10.7 (0.084)	<1.0 (<0.001)	2.0 (0.003)
Collagenase	11.9 (0.426)	8.1 (0.064)	4.3 (0.002)	8.8 (0.014)
First pancreatin	16.0 (0.572)	17.7 (0.139)	9.6 (0.005)	10.8 (0.017)
Subsequent enzymes combined	23.0 (0.822)	22.7 (0.178)		13.5 (0.022)
HCl	1.1 (0.039)	1.5 (0.012)		
NaOH	1.9 (0.067)	2.2 (0.018)		
Unextracted residue	2.2 (0.080)	1.3 (0.010)	15.8 (0.008)	4.3 (0.007)

Table notes: Extraction protocol is outlined in Figure 5. Full protocol performed only with liver and kidney. Blank spaces indicate fractions not generated for given tissues.

Table 12. Distribution of radioactive residues in cow milk and fat.

Fraction	Recoveries in % TRR (ppm) from tissue [TRR in ppm]:			
	Omental fat [1.278]	Renal fat [0.840]	Subcutaneous fat [0.305]	Milk [0.147]
Hexane	4.2 (0.054)	0.8 (0.007)	<1.0 (<0.003)	1.53 (0.002)
Combined acetonitrile	77.5 (0.991)	77.5 (0.651)	64.1 (0.195)	88.6 (0.131)
Acetonitrile	6.1 (0.078)	6.3 (0.053)		
Acetonitrile: water			12.4 (0.038)	
Unextracted residue	4.3 (0.055)	3.5 (0.030)	19.7 (0.060)	4.7 (0.007)

Table notes: Extraction protocol is outlined in Figure 5. Full protocol performed only with liver and kidney; protocols for milk and fat are summarized in the text. Blank spaces indicate fractions not generated for given tissues.

Residue Analysis

Extracts were analyzed by thin layer chromatography (TLC) using silica plates, developed in one dimension in any of four different solvents: hexane:dichloromethane (10:3), hexane:acetone (6:1), toluene:acetone:acetonitrile (100:10:1), or chloroform:ethyl acetate (3:1). Extracts were also analyzed by HPLC, using a Zorbax ODS column, developed with a gradient from 10% acetonitrile, 0.1% aqueous glacial acetic acid as Solvent A, to 90% acetonitrile, 0.1% aqueous glacial acetic acid as Solvent B. Residues were assigned based on comparisons with mobilities of standards.

Combined acetonitrile extracts from milk showed a single strong peak when analyzed by HPLC or any of the TLC systems; this peak was assigned to endosulfan sulfate. Similar results were seen during analysis of combined acetonitrile extracts from fat samples. The acetonitrile extracts from omental and renal fat (Table 12) were also analyzed by HPLC. Attempts to analyze the acetonitrile:water extract from subcutaneous fat by TLC were unsuccessful because the sample contained oily and particulate matter. For all the fat samples, endosulfan sulfate was the only residue assigned.

Combined acetonitrile extracts from loin muscle were also analyzed by TLC and HPLC; other extracts were not analyzed because of low levels of radioactivity. TLC showed a strong peak consistent with the mobility of endosulfan sulfate. HPLC analysis indicated a peak for the sulfate, and a smaller peak assigned to alpha endosulfan. Combined acetonitrile extracts from heart were also analyzed. TLC indicated a strong peak consistent with the sulfate, and a smaller peak with the same mobility as the lactone (Figure 6). On the basis of HPLC, residues in heart were assigned to the sulfate, the lactone, and smaller amounts to alpha endosulfan.

Combined acetonitrile extracts from liver were also analyzed by TLC and HPLC. A strong peak was assigned to endosulfan sulfate, but a smaller peak was designated polar residues because of a short retention time with HPLC, and retention at the origin with TLC. On the basis of HPLC and TLC analysis of the methanol extract, a small peak was assigned to alpha endosulfan, and a larger peak to polar residues. In subsequent liver extracts, peaks assigned to polar residues were more significant. Consequently, enzyme and aqueous fractions were combined, hydrolyzed in HCl, and then extracted with ethyl acetate (Figure 5). When the ethyl acetate fraction was analyzed by TLC, and HPLC, a strong polar peak remained. Smaller peaks were assigned to alpha endosulfan, the diol, the ether, and the lactone (Figure 6). Analysis of extracts from kidney gave similar results with a major portion of residues assigned as polar compounds, and the ethyl acetate fraction gave additional peaks assigned to beta endosulfan, the sulfate, and the hydroxy ether. The assignment of residues is summarized in Table 13 for liver and kidney, and in Table 14 for other cow tissues:

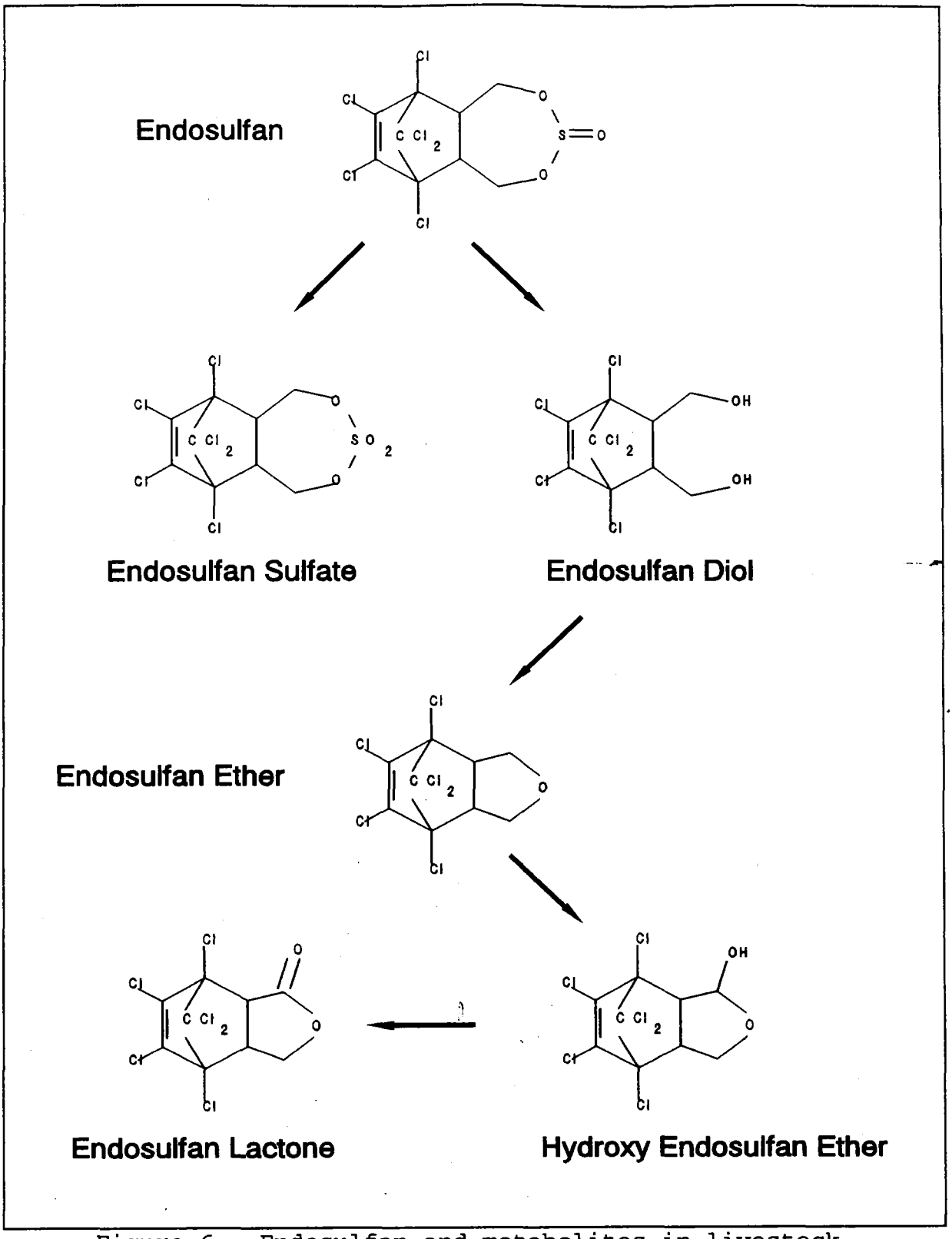

Figure 6. Endosulfan and metabolites in livestock.

Table 13. Residue assignment in cow liver and kidney.

Residue	% TRR (ppm) in Tissue [100% TRR, in ppm]	
	Liver [3.572]	Kidney [0.785]
Alpha Endosulfan	0.2 (0.007)	
Endosulfan Sulfate	27.2 (0.972)	8.3 (0.065)
After acid hydrolysis:		
Alpha Endosulfan	2.5 (0.089)	
Beta Endosulfan		3.1 (0.024)
Endosulfan Sulfate		4.3 (0.034)
Endosulfan Diol	6.5 (0.232)	5.0 (0.039)
Endosulfan Ether	6.8 (0.243)	2.1 (0.016)
Hydroxy Endosulfan Ether		4.8 (0.038)
Endosulfan Lactone	9.2 (0.329)	6.9 (0.054)
Total Identified	52.4 (1.872)	34.5 (0.271)
Polar	35.5 (1.268)	51.6 (0.405)

Table note: Blank spaces indicate residues not detected. See Figure 6 for structures.

Table 14. Residue assignment in other cow tissues.

Residue	% TRR (ppm) in Tissue [100% TRR, in ppm]					
	Heart [0.161]	Loin Muscle [0.052]	Omental fat [1.278]	Renal fat [0.840]	Subcutaneous fat [0.305]	Milk [0.147]
Alpha Endosulfan	2.2 (0.004)	15.1 (0.008)				
Endosulfan Sulfate	14.2 (0.023)	50.7 (0.026)	82.1 (1.049)	83.8 (0.704)	67.8 (0.207)	88.6 (0.130)
Endosulfan Lactone	29.5 (0.047)					

Table note: Blank spaces indicate residues not detected. See Figure 6 for structures.

CBRS Comments

The Registrant did not provide data on stability of residues during frozen storage during the study. However, the present submission reported that the cow was sacrificed on February 20, 1995, and the last analyses were completed in September 1995. It therefore seems likely that analysis of most tissues was completed within 6 months of sacrifice, and storage stability data are not required.

In subcutaneous fat, unextracted residues represented greater than 0.05 ppm and greater than 10% of TRR (Table 12). However, in omental and renal fat, over 80% of TRR was identified as endosulfan sulfate, and this metabolite represented nearly 70% of the residue in subcutaneous fat. Further work in fat is not required.

Residue identification is not required in heart, but the Registrant identified 46% of TRR in this organ. In liver and kidney, major portions of TRR were characterized as polar compounds. However, this characterization followed extensive attempts to cleave metabolites that may have been conjugated. Further work in liver and kidney therefore is not required. The considerations above lead to the following comments:

Conclusion 8: In milk, 89% of total residues were identified as endosulfan sulfate. In muscle, 66% of total residues were identified as alpha endosulfan and the sulfate. Although data are not required for heart, 46% of total residues in this organ were identified as alpha endosulfan, endosulfan sulfate, and endosulfan lactone. (Table 14 and Figure 6) In each of these tissues, individual unidentified residues each represented less than 10% TRR and/or less than 0.05 ppm.

Conclusion 9: In fat samples, 68% to 84% of total residues were identified as endosulfan sulfate (Table 14). Considering all the data from omental, renal, and subcutaneous fat, further work on these samples is not required.

Conclusion 10: In liver, 52% of total residues were identified as alpha endosulfan, endosulfan sulfate, and potential conjugates of endosulfan or specific metabolites. In kidney, 34% of total residues were identified as the sulfate and potential conjugates of endosulfan or specific metabolites. (see Table 13 and Figure 6) In liver and kidney, 30-50% of total residues were designated as polar compounds, but this assignment followed extensive attempts to cleave metabolites that may have been conjugated. With these considerations and the extensive identification of residues in milk, muscle, and fat, further work on liver and kidney is not required.

The submitted data leads to the following comment:

Conclusion 11: All of the metabolites identified contained intact chlorinated rings, including all labeled carbons, even after harsh treatments to cleave conjugates. Identified metabolites represented 50% TRR in liver and higher portions in fat, muscle, and milk (89% TRR). This information indicates the stability of labeled rings. With this consideration, and Conclusions 8 through 10 above, the nature of the residue in cow is adequately understood.

HEN

Livestock Procedures

The following study was submitted:

Endosulfan: Distribution, Elimination and the Nature of the Metabolite Residues in the Eggs and Edible Tissues of the Laying Hen, AgrEvo USA Company, Report A56354, March 26, 1996 (MRID 44099102).

The performing laboratory for animal dosing and laboratory analysis was AgrEvo UK Limited, Toxicokinetics, Essex, England. Test substance was [^{14}C]endosulfan, labeled at the 6, 7, 8, 9, and 10 ring positions; the ratio of alpha to beta isomers was approximately 70:30 (Figure 2 above shows the labels on the alpha isomer; the same atoms were also labeled on beta isomer in the test substance). Specific activity was 4522 dpm/μg, and radiochemical purity was 97%.

Test substances were placed in gelatin capsules and six laying hens of strain Lohmann Brown were dosed once daily at approximately 11 ppm in the diet, for 12 consecutive days. Eggs were collected twice daily. Hens were sacrificed approximately 23 h after receiving the last dose. Breast and thigh muscle, liver, subcutaneous and abdominal fat, skin, and any unlaid developing eggs were removed and stored at approximately -20°C until analysis. Urine and feces were also collected to account for the distribution of the administered dose.

Laboratory Analysis

To determine total radioactive residues (TRR) in tissues, samples were minced or homogenized and aliquots were placed in solubilizing solution, a mixture of aqueous sodium hydroxide, Triton detergent, and methanol. The mixture was neutralized with HCl, then added to scintillation cocktail, and radioactivity was determined by liquid scintillation counting (LSC). Residues are summarized in Table 15. Maximum average residues were observed

on day 12 for egg yolks and day 6 for egg whites. The performing laboratory reported that radioactivity in urine and feces accounted for about 87% of the administered dose.

Table 15. Total radioactive residues in poultry tissues.

Tissue	TRR, ppm
Liver	0.466
Skin	0.689
Muscle	0.028
Abdominal fat	0.974
Subcutaneous fat	0.875
Undeveloped eggs	0.768
Egg yolks	0.899
Egg whites	0.013

Table notes: Residues are averages from six hens. For egg yolks and whites, values are maximum average residues during 12 days of dosing.

Extraction protocols were similar, but not identical, to the extractions of cow tissues (Figure 5). The most extensive protocol for hen was with liver. Tissue samples pooled from each hen were homogenized in hexane, extracted by stirring overnight, filtered, and the pellet was extracted in hexane again. The combined hexane extract was partitioned with acetonitrile. The pellet from hexane extraction was resuspended and extracted in acetonitrile. The solution was filtered and the filtrate was combined with other acetonitrile extracts. The pellet remaining from acetonitrile extraction was extracted in acetonitrile:water (70:30).

The pellet from acetonitrile:water extraction was resuspended and extracted in methanol. The remaining pellet was then extracted in water. The pellet from water extraction was treated sequentially with the enzymes collagenase, pancreatin (containing amylase, trypsin, lipase, ribonuclease, and protease), and then proteinase K. In a separate analysis, the enzyme fractions were pooled. An aliquot was refluxed in 6 N HCl, then partitioned with ethyl acetate:hexane into organic and aqueous phases.

Other tissues were extracted using similar but less extensive protocols. With egg yolks, the combined acetonitrile extract included the acetonitrile:water extract. An aliquot of the combined acetonitrile extract was treated with 1 N HCl to cleave

conjugates. Yolks were extracted through the water fraction, and the remaining pellet was treated with proteinase K. The water and proteinase K fractions were then pooled, refluxed in HCl, and partitioned into organic and aqueous phases.

Skin was extracted through the acetonitrile:water step, then all extracts containing acetonitrile were pooled, and partitioned into hexane and acetonitrile extracts. An aliquot of the acetonitrile extract was treated with 1 N HCl. Subcutaneous fat was extracted with hexane and acetonitrile, and the remaining pellet was extracted with water. Abdominal fat was extracted through the combined acetonitrile extract; the hexane extract was partitioned into hexane and acetonitrile extracts. Muscle was extracted through the acetonitrile:water step. Egg whites were extracted in acetonitrile only; because of low levels of radioactivity, neither the acetonitrile fraction nor the unextracted residues were analyzed further. TRR was higher in egg yolks than in undeveloped eggs, and the latter tissue was not extracted. Table 16 summarizes the distribution of radioactive residues in poultry tissues.

Residue Analysis

Extracts were analyzed by thin layer chromatography (TLC) using silica plates, developed in one dimension in any of four different solvents: hexane:dichloromethane (10:3), hexane:acetone (6:1), toluene:acetone:acetonitrile (100:10:1), or chloroform:ethyl acetate (3:1). Extracts were also analyzed by HPLC, using a Zorbax ODS column, developed with a gradient from 10% acetonitrile, 0.1% acetic acid as Solvent A, to 90% acetonitrile, 0.1% acetic acid as Solvent B. Residues were assigned based on comparisons with mobilities of standards.

Table 16. Distribution of radioactive residues in poultry tissues.

Fraction	Recoveries in % TRR (ppm) from tissue [TRR in ppm]:						
	Liver [0.466]	Egg yolks [0.853]	Skin [0.689]	Abdominal fat [0.974]	Subcutaneous fat [0.875]	Muscle [0.028]	Egg whites [0.013]
Hexane	2.6 (0.012)	0.6 (<0.01)	5.0 (0.034)	0.6 (0.005)	0.5 (0.005)	0.4 (<0.001)	
Acetonitrile				2.7 (0.026)			
Combined acetonitrile	63.3 (0.295)	74.8 (0.637)	85.0 (0.586)	92.3 (0.900)	91.2 (0.798)	56.4 (0.016)	64.9 (0.008)
Acetonitrile: water	5.3 (0.025)					7.8 (0.002)	
Methanol	5.1 (0.024)	2.3 (0.020)					
Water	3.5 (0.016)	0.5 (0.004)			6.3 (0.055)		
Combined enzyme digests	8.3 (0.039)	9.3 (0.080)					
Unextracted residue	8.6 (0.040)	7.8 (0.067)	5.6 (0.039)	3.0 (0.029)	1.8 (0.016)	35.6 (0.010)	35.1 (0.005)

Table notes: Extraction protocol was similar to Figure 5 and is described in the text. The most extensive extractions were with liver and egg yolks. Blank spaces indicate fractions not generated for given tissues.

As noted above, TRR in egg whites was less than 0.02 ppm, and extracts were not analyzed. TRR in muscle was 0.028 ppm. When analyzed by HPLC, combined acetonitrile extracts from muscle showed a strong peak assigned to endosulfan sulfate; smaller peaks were assigned to alpha and beta endosulfan, the lactone, and to polar compounds. The same compounds were assigned based on analysis of combined acetonitrile extracts from abdominal and subcutaneous fat.

Analysis of the combined acetonitrile extract from skin showed the same peaks assigned in other tissues (alpha and beta parent, the sulfate, and the lactone), plus a polar peak assigned to 12.8% TRR. When the combined acetonitrile extract was hydrolyzed in HCl, the polar peak disappeared, and new peaks appeared corresponding to the diol, the ether, and the hydroxy ether (Figure 6). The acid hydrolysis also eliminated the peaks assigned to alpha and beta parent, so the portion of TRR represented by each of the new metabolites was not assigned.

Analysis of the combined acetonitrile extract from liver gave a strong peak assigned to endosulfan sulfate, and smaller peaks assigned to alpha and beta parent, the diol, and the lactone; a small peak (3% TRR) assigned to polar compounds was also seen. Polar peaks were also seen in the more aqueous extracts that followed. Acid hydrolysis of the combined enzyme digests was not successful in increasing residue identification. However, each polar component represented less than 0.03 ppm.

Analysis of the combined acetonitrile extract from egg yolks gave a strong peak assigned to the sulfate and smaller peaks assigned to alpha and beta parent and the lactone, plus a polar peak assigned to 17.8% TRR. When the combined acetonitrile extract was hydrolyzed in HCl, the polar peak disappeared, the lactone peak increased, and new peaks appeared corresponding to the diol, the ether, and the hydroxy ether. As was the case with skin extract, acid hydrolysis of the yolk extract also eliminated the peaks assigned to alpha and beta parent, and the portion of TRR represented by each of the new metabolites was not assigned. Analysis of the more aqueous extracts from yolks, including acid hydrolysis of combined water and proteinase extracts, did not significantly increase the assignment of residues. Table 17 summarizes the assignment of residues in poultry tissues:

Table 17. Residue assignment in poultry tissues.

Residue	% TRR (ppm) in Tissue [100% TRR, in ppm]					
	Egg yolks [0.853]	Skin [0.689]	Abdominal fat [0.974]	Subcutaneous fat [0.875]	Liver [0.466]	Muscle [0.028]
Alpha Endosulfan	4.7 (0.040)	11.7 (0.081)	16.8 (0.164)	16.2 (0.142)	1.0 (0.005)	6.5 (0.002)
Beta Endosulfan	1.3 (0.011)	4.8 (0.033)	7.8 (0.076)	8.9 (0.078)	1.6 (0.007)	4.4 (0.001)
Endosulfan Sulfate	46.4 (0.396)	51.3 (0.353)	65.4 (0.637)	61.1 (0.535)	45.6 (0.212)	35.8 (0.010)
Endosulfan Diol					4.2 (0.020)	
Endosulfan Lactone	1.8 (0.015)	4.5 (0.031)	5.0 (0.049)	5.0 (0.044)	6.3 (0.029)	3.5 (0.001)
After acid hydrolysis:						
Endosulfan Diol	17.8 (0.152)	12.8 (0.088)				
Endosulfan Ether						
Hydroxy Endosulfan Ether						
Endosulfan Lactone						
Total Identified	72.0	85.1	95.0	91.2	58.7	50.2

Table note: Blank spaces indicate residues not detected. See Figure 6 for structures. TRR in egg whites < 0.02 ppm. Each individual unidentified residue represented less than 10% TRR and/or 0.05 ppm.

CBRS Comments

The Registrant did not provide data on stability of residues during frozen storage during the study. However, the present submission reported that hens were sacrificed on March 7, 1995, and the last analyses were completed in September 1995. It therefore seems likely that analysis of most tissues was completed within 6 months of sacrifice, and storage stability data are not required.

Residue identification was not required in skin, but the Registrant identified 85% of TRR in this organ. The considerations above lead to the following comments:

Conclusion 12: Residues identified in poultry tissues ranged from 50% TRR in muscle to 95% TRR in fat; residues identified included alpha and beta endosulfan, endosulfan sulfate, endosulfan diol, endosulfan lactone, and potential conjugates of specific metabolites (Table 17 and Figure 6). In all required poultry tissues, individual unidentified residues each represented less than 10% TRR and/or less than 0.05 ppm.

Conclusion 13: All of the residues identified contained intact chlorinated rings, including all labeled carbons, even after treatments to cleave conjugates. Identified metabolites represented 50% TRR in muscle and higher portions in other tissues. This information indicates the stability of labeled rings. With this consideration and Conclusion 12, the nature of the residue in poultry is adequately understood.

Recommendation: In accordance with Conclusions 8 through 13, Guideline 860.1300 is satisfied for livestock. The data submitted will be presented to the HED Metabolism Committee for a determination of the residues to be regulated in livestock.

cc:Circ, Abbotts, RF, Endosulfan List A File, SF,
Sepehr Haddad (SRRD)
RDI:ARRathman:3/28/97:RBPerfetti:4/2/97
7509C:CBII-RS:JAbbotts:CM-2:Rm805A:305-6230:4/2/97
JA23\endosulf.3

ENDOSULFAN (CASE 14/CODE 079401)
UNOFFICIAL RESIDUE CHEMISTRY DATA SUMMARY THROUGH 4/2/97
(AGENCY USE ONLY)[1]

REASSESSMENT OF U.S. TOLERANCES AND POTENTIAL FOR CODEX HARMONIZATION[2]

Guideline Number and Topic[3]	Are data requirements satisfied?	MRID(s)[4]
860.1200 Directions for use	N	
860.1300 Nature of the Residue, Plants	Y[5]	44082701,44082702, 44099101
860.1300 Nature of the Residue, Livestock	Y[6]	44082703,44099102
860.1340 Residue Analytical Methods, Plants	N	
860.1340 Residue Analytical Methods, Livestock	N	
860.1380 Storage Stability	N	
860.1500 Crop Field Trials	--[7]	
860.1500 Root and Tuber Vegetables Group		
Carrots	Y	
Potatoes [see 860.1520]	Y	
Sugar beets [see 860.1520]	Y	
Sweet potato	Y	
860.1500 Leaves of Root and Tuber Vegetables		
Sugar beet tops	Y	
Turnip tops	Y	
860.1500 Leafy Vegetables (except Brassica)		
Celery	N	
Lettuce (leaf)	N	
Lettuce (head)	N	
Spinach	Y	
860.1500 Brassica Leafy Vegetables Group		
Broccoli	N	
Brussels sprouts	Y	
Cabbage	Y	
Cauliflower	Y	
Collards	Y	
Kale	Y	
Mustard greens	Y	
860.1500 Legume Vegetables (succulent/dried)		
Beans (succulent and dried)	Y	
Peas (succulent and dried)	N	
Soybeans [see 860.1520]	N	
860.1500 Foliage of Legume Vegetables		
Bean vines and hay	Y	
Cowpea forage and hay	N	
Pea vines and straw	Y	
Soybean forage and hay	N	

ENDOSULFAN (CASE 14/CODE 079401)
UNOFFICIAL RESIDUE CHEMISTRY DATA SUMMARY THROUGH 4/2/97
(AGENCY USE ONLY)[1]

REASSESSMENT OF U.S. TOLERANCES AND POTENTIAL FOR CODEX HARMONIZATION[2]

Guideline Number and Topic[3]	Are data requirements satisfied?	MRID(s)[4]
860.1500 Fruiting Vegetables Group		
Eggplant	N	
Peppers	Y	
Tomatoes [see 860.1520]	N	
860.1500 Cucurbit Vegetables Group		
Cucumbers	N	
Melons	N	
Pumpkins	N	
Squash (summer/winter)	N	
Watermelons	N	
860.1500 Pome Fruits Group		
Apples [see 860.1520]	Y	
Pears	Y	
860.1500 Stone Fruits Group		
Apricots	Y	
Cherries	N	
Nectarines	Y	
Peaches	Y	
Plums (fresh prunes) [see 860.1520]	Y	
860.1500 Small Fruits and Berries Group		
Blueberries	Y	
Grapes [see 860.1520]	Y	
Strawberries	Y	
860.1500 Tree Nuts Group		
Almonds	Y	
Filberts	Y	
Macadamia	Y	
Pecans	Y	
Walnuts	Y	
860.1500 Cereal Grains Group		
Barley [see 860.1520]	N	
Corn (field) [see 860.1520]	N	
Corn (sweet) [see 860.1520]	N	
Oats [see 860.1520]	N	
Rye [see 860.1520]	N	
Wheat [see 860.1520]	Y	

ENDOSULFAN (CASE 14/CODE 079401)
UNOFFICIAL RESIDUE CHEMISTRY DATA SUMMARY THROUGH 4/2/97
(AGENCY USE ONLY)[1]

REASSESSMENT OF U.S. TOLERANCES AND POTENTIAL FOR CODEX HARMONIZATION[2]

Guideline Number and Topic[3]	Are data requirements satisfied?	MRID(s)[4]
860.1500 Forage, Fodder, and Straw of Cereal Grains		
Barley forage and straw	N	
Corn (field and pop) forage and fodder	N	
Corn (sweet) forage	N	
Oats forage and straw	N	
Rye forage and straw	N	
Wheat forage and straw	N	
860.1500 Non-grass Animal Feeds		
Alfalfa [see 860.1520]	N	
860.1500 Miscellaneous Commodities		
Artichokes	N	
Cottonseed [see 860.1520]	Y	
Pineapple [see 860.1520]	Y	
Rapeseed	Y	
Safflower seed [see 860.1520]	Y	
Sunflower [see 860.1520]	Y	
Tea (dried leaves)	Y	
Tobacco	Y	
Watercress	N	
860.1520 Processed Food/Feed	--[8]	
Apples	Y	
Barley	N	
Beans (succulent/dried)	N/A	
Corn, Field	N	
Corn, Sweet	N	
Cottonseed	N	
Grapes	N	
Oats	N	
Pineapple	N	
Plums	Y	
Potato	N	
Rapeseed	N	
Rye	N	
Safflower	N	
Soybeans	N	
Sugar beets	N	
Sunflower	N	
Tomato	N	
Wheat	N	
860.1480 Meat/Milk/Poultry/Eggs	Reserved[9]	
860.1400 Potable Water	Reserved	

ENDOSULFAN (CASE 14/CODE 079401)
UNOFFICIAL RESIDUE CHEMISTRY DATA SUMMARY THROUGH 4/2/97
(AGENCY USE ONLY)[1]

REASSESSMENT OF U.S. TOLERANCES AND POTENTIAL FOR CODEX HARMONIZATION[2]

Guideline Number and Topic[3]	Are data requirements satisfied?	MRID(s)[4]
860.1400 Fish	Reserved	
860.1400 Irrigated Crops	N/A	
860.1460 Food Handling Establishments	N/A	
860.1850 Confined Rotational Crops Reduction of Residues	N[10]	

[1] Registration Standard issued 4/82. Reregistration Standard Update to the Residue Chemistry Chapter issued 8/9/90. This summary is unofficial and subject to correction.

[2] Codex MRLs are established for combined residues of alpha-Endosulfan, beta-Endosulfan, and Endosulfan sulfate, the same residues in the CFR tolerance expression, for numerous commodities.

[3]N/A = Guideline requirement not applicable.

[4]MRIDs that were reviewed in the current submission are designated in shaded type.

[5] CBRS 17547, 17855, 4/2/96, J. Abbotts. MRID 44082702: The nature of the residue in lettuce is adequately understood. MRID 44099101: With the data from leaves, the nature of the residue in cucumber is adequately understood. MRID 44082701: With the combined data on fruit and leaves, the nature of the residue in apples is adequately understood. This Guideline is satisfied for crops. The data submitted, including data on leaves, will be presented to the HED Metabolism Committee for a determination of the residues to be regulated in plants.

[6] CBRS 17547, 17855, 4/2/97, J. Abbotts. MRID 44082703: The nature of the residue in cow is adequately understood. MRID 44099102: The nature of the residue in poultry is adequately understood. This Guideline is satisfied for livestock. The data submitted will be presented to the HED Metabolism Committee for a determination of the residues to be regulated in livestock.

[7] Conclusions on field trial data may change with data on plant metabolism, analytical method, or storage stability.
Memo, 9/24/96, J. Abbotts: DRES reported that the following published tolerances are not being supported for reregistration: alfalfa, artichokes, barley, oats, succulent peas, rye, safflower, sugar beets, sunflower, watercress, and wheat. CBRS recommends that these tolerances be revoked.
Greybeard Committee, 1/8/97: CBRS has no objection to a deadline extension to 5/97 for this Guideline.

[8] Greybeard Committee, 1/8/97: CBRS has no objection to a deadline extension to 5/97 for this Guideline.

[9] Greybeard Committee, 1/8/97: CBRS rejects the request to waive residue chemistry requirements for Endosulfan cattle ear tags; formal submissions are required.

[10] Greybeard Committee, 1/8/97: CBRS has no objection to a deadline extension to 5/97 for this Guideline.

cc: Endosulfan List A Reregistration Standard File
14.1

List A

Note to file: Endosulfan and the Greybeard Decision of 01/08/97.

Mr. Fred Smith of SRA Int (202-728-1400) inquired for Fermenta on the ear tag use. He indicated that the registrant will drop the use rather than fund additional studies.

He asked if a formal submission of the tag dissipation study and a summary of all exisiting metabolism studies submitted to EPA would suffice. I indicated that this was possible, not necessarily probable. The animal metabolism studies would need to show unambiguously that endosulfan or metabolites of concern do no bioaccumulate.

Mr. Smith indicated that the registrant will make a formal submission of the tag dissipation study and a summary with references of all EPA-accepted (plus other?) animal metabolism studies for endosulfan as a response to the Greybeard decision.

2/13/97

Greybeard Committee: Endosulfan
DP Barcode None. 01/08/97.

This is a time extension request for GLN 165-1, or OPPTS 860.1850, and for GLN 171-4(k/l), or 860.1500/.1520, origin and date unknown, forwarded by S. Haddad of SRRD.

A time extension is requested for OPPTS 860.1850 from the due date of 05/96 to 05/97 and is based on (1) time required to prepare the radiolabeled material and (2) inability to initiate study until 02/95, because notice of requirement was not received until late 1994. Thus, the harvest for the 365 DAT planting could not occur until the May - September 1996 period. Additional time is then needed to analyze the crop samples and write a report.

CBRS has no objection to the time extension until 05/97 for OPPTS 860.1850, but is aware that the granting of time extensions is the prerogative of SRRD.

A time extension is requested for OPPTS 860.1500 and 860.1520 from 05/96 until 05/97 and is based on (1) loss of 1994 growing season because of arrival of DCI late in growing year and (2) need to decide upon crops to support. Trials will be conducted in the 1995 season and in early 1996. Progress reports will be submitted.

CBRS has no objection to the time extension until 05/97 for OPPTS 860.1500 and OPPTS 860.1520, but recognizes that the granting of time extensions is the prerogative of SRRD.

cc: Subject File, Registration Standard File, Greybeard File, M. Metzger (RCAB).

SRF:01/08/97:GB97-1.

Greybeard Committee: Endosulfan
DP Barcode None. 01/08/97.

Fermenta has requested (date ?) a waiver from all residue chemistry data requirements for endosulfan cattle ear tags. The request is based on (1) slow rate of release from the tags; (2) no substantial bioaccumulation; and (3) endosulfan does not persist in animal tissue and is readily eliminated.

The registrant presents summary data for ear tag depletion and calculates a release of 15 mg a.i./day, or 0.038 mg/kg bw/day. The registrant speculates that only a small amount of the released pesticide will penetrate the haircoat. Some summary fat and milk residue data are presented for cows pastured on treated grasses.

The evidence presented does not adequately support the waiver request, and *CBRS rejects the request to waive residue chemistry data for endosulfan cattle ear tags*. The registrant should make formal submissions of nature of the residue studies in ruminants (oral administration) to support the contention of lack of bioaccumulation and of tag dissipation studies to show the loss of active ingredient with time. Alternatively, the registrant must submit nature of the residue studies and magnitude of the residue studies for the dermal use of endosulfan on ruminants.

cc: Subject File, Registration Standard File, Greybeard File, M. Metzger (RCAB).
srf:01/08/97:gb97-6.

13544

R111501

Chemical:	**Endosulfan**
PC Code:	**079401**
HED File Code	**11000 Chemistry Reviews**
Memo Date:	**04/02/1997**
File ID:	**DPD229661; DPD234815**
Accession Number:	**412-05-0099**

HED Records Reference Center
08/11/2005