



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

JAN 29 1991

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Plant metabolism and processing study requirements for re-registration of pendimethalin. (MRID# 41469901; DEB#'s 6570, 6603, 6604, 7153; HED Project #'s 1-0006, 0-1060, 0-1127A, 0-1127)

FROM: Richard Loranger, Ph.D., Chemist *R. Loranger*
Chemistry Branch I, HED (H7509C)

and
Randolph B. Perfetti, Ph.D., Chemist *R.B. Perfetti*
Chemistry Branch II, HED (H7509C)

THRU: Richard D. Schmitt, Ph.D., Chief *Richard D. Schmitt*
Chemistry Branch I, HED (H7509C)
and
Edward Zager, Acting Chief *Edward Zager*
Chemistry Branch II, HED (H7509C)

TO: Reto Engler, Ph.D., Chief
Science Analysis and Coordination Branch
Health Effects Division (H7509C)
and
Lois Rossi, Chief
Reregistration Branch
Special Review and Reregistration Division (H7508C)

Attached is a review of a soybean metabolism study submitted by American Cyanamid Corporation in response to the Pendimethalin Registration Standard. In addition, the review discusses plant metabolism studies that are resubmitted by American Cyanamid as part of their argument as to why no additional plant metabolism studies should be required for this herbicide. The review also addresses the registrant's argument against the need for additional processing studies.

The review was prepared by Dynamac Corporation under the supervision of the Chemistry Branches, HED. The review has undergone secondary review in the Chemistry Branches and has been revised to reflect the Branches' policy.

Please refer to the Conclusions and Recommendations in the attached review for the additional plant metabolism and processing data required for reregistration of pendimethalin. If you need additional input, please advise.

RECEIVED
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Attachment 1: Review of Pendimethalin-Registrant's Response
to Residue Chemistry Data Requirements

Attachment 2: July 25, 1989 R. Schmitt Memo

cc with Attachments 1 & 2: R. Loranger, R. Perfetti, Circu,
Pendimethalin Reg. Std. File, Pendimethalin SF, J. Burrell
(PIB/FOD), C. Furlow (PIB/FOD), Dynamac

cc without Attachments: P. Fenner-Crisp (HED), RF

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PENDIMETHALIN

REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

Task - 4

BACKGROUND

The Pendimethalin Guidance Document dated 3/29/85 concludes that the qualitative nature of the residue in plants is not adequately understood and additional data are required. As of 3/19/90, the date of the Pendimethalin Registration Standard Update, the requirements for data depicting the distribution and metabolism of [¹⁴C]pendimethalin in representative food crops remained outstanding.

In response to the data requirements, American Cyanamid (DEB Nos. 6570/6603/6604/7153) has submitted (cover letter dated 4/18/90) four previously submitted studies (MRIDs 00074621, 00093698, 00106795, and EPA ID #GS0187-002) depicting the nature of the residue in sweet corn, peanut hulls, and potato tubers, and one new study (1987; MRID No. 41469901) depicting the nature of the residue in soybeans. The registrant disagreed with the residue and metabolism review in the Pendimethalin Residue Chemistry Chapter dated 7/20/84, and cited older Agency reviews (dated 4/29/82 and 7/15/82) stating that the nature of residue in plants and animals was adequately understood and no new studies were required. American Cyanamid contends that the available data are sufficient and that no new plant metabolism data should be required.

In the 4/18/90 cover letter American Cyanamid also states that no further processing work should be necessary since additional studies (MRID# 40185102) show pendimethalin is not present at 2 ppb in corn grain and therefore could not concentrate above the 50 ppb tolerance level for pendimethalin in corn grain. Dietary Exposure Branch has reviewed these studies (D. Edwards, 9/8/89) and concluded that the tolerance for grain should be lowered to 2 ppb and a processing study conducted using corn grain treated at the highest practical exaggerated rate.

CONCLUSIONS:

The qualitative nature of the residue in plants is not adequately understood. The conclusions arrived at in the Agency's 4/29/82 and 7/15/82 Residue Chemistry Branch reviews were based on Guideline requirements then in effect (1982) at EPA. The Guidelines for metabolism studies in 1982 were not specific and did not contain criteria for characterization of metabolites and ¹⁴C-residues isolated in these studies. In addition, the Guidelines in effect in 1982 did not contain criteria for the characterization of residues designated as unextractable or bound. The resubmitted studies are summarized below along with a

discussion of the newly submitted study of soybean metabolism. The available data are insufficient for the following reasons:

1. In the studies on sweet corn, the low application rates (0.75x) and the use of 3,4-methyl-, 4-methyl-, and N-ethylpropyl-labeled [¹⁴C]pendimethalin instead of uniformly, ring-labeled test material resulted in levels of ¹⁴C-residues too low for adequate characterization. Up to 29% of the total radioactivity in immature plants, and up to 55% of the total radioactivity in mature grain and cobs were not characterized.
2. Less than 5% of the total radioactive residues (TRR) in peanut hulls were characterized and no data were reported for ¹⁴C-residues in peanut nutmeats and foliage.
3. In studies on potato tubers, application of 4-methyl-labeled [¹⁴C]pendimethalin instead of uniformly, ring-labeled test material resulted in levels of ¹⁴C-residues too low for adequate characterization. In addition, large amounts of co-extractable components interfered with the accurate quantitation of the residues.
- 4a. Only 5% (0.007-0.15 ppm) of TRR in immature soybean plants were identified, and only three reference standards were used, although 25 different components with ¹⁴C-activity were isolated.
- 4b. The reported incorporation of ¹⁴C-residues in soybean matrices into crude lignin, crude cellulose, and protein was not supported by data from hydrolytic procedures.
- 4c. ¹⁴C-Residues in soybean hulls (0.18 ppm) were not characterized.
5. With regard to the need for processing studies, the Agency has reconsidered the situation for corn and concluded that a corn processing study is not required, provided additional residues of concern are not found in the additional plant metabolism studies. However, in the absence of a processing study for corn, processing studies for all other oilseed crops on which pendimethalin is registered are required per the current Chemistry Branch policy. The pertinent crops should be treated in the field at the highest practical exaggerated rate and then processed into those fractions cited in the Residue Chemistry Guidelines. If any particular crop can be treated at rates higher than the maximum theoretical concentration factor and no detectable residues of concern are found in the seed, the processing study may be waived for that crop. Chemistry Branch emphasizes that the intentional setting of a tolerance on a raw agricultural commodity (e.g., corn grain) high enough such that

residues in a processed commodity (e.g., corn oil) will not exceed that tolerance will not be accepted in the future.

RECOMMENDATIONS

The registrant should be informed that additional processing studies are required as outlined in Conclusion 5 and that the plant metabolism data base for pendimethalin is not adequate. Although the available studies indicate that low levels of radioactivity are taken up from the soil into aerial parts of plants, these studies were conducted with pendimethalin radiolabeled in side chains as opposed to in the phenyl ring. In addition, most of the studies were conducted using application rates lower than the maximum permitted on product labels.

Additional metabolism studies are required in which pendimethalin radiolabeled with ^{14}C in the phenyl ring is applied to plants at rates equal to at least the maximum rates on product labels. Provided significant phytotoxicity does not occur, even higher application rates are preferred to increase the level of radioactivity available for analysis. One study should be conducted on sweet corn with examinations of vegetative parts and grain from (1) plants treated pre-emergence and (2) plants treated post-emergence as late in the growing season as practical. A second study is needed on a plant in which the edible portion grows in the soil, i.e., potatoes or peanuts.

In these additional metabolism studies data depicting all terminal residue components in commodities at the time of harvest should be expressed as the percentage of the total recovered radioactivity and the concentration (ppm). For the degree of characterization required in these studies, the registrant should refer to the "trigger values" discussed in the attached July 25, 1989 memo (published in the 12/24/89 Phase 3 Technical Guidance Package). Confirmation of the identities of the residues using a suitable method such as MS, HPLC, GLC, TLC, etc. is also required. Representative samples from these studies must also be analyzed by the residue analytical methods developed for data collection and tolerance enforcement to ascertain that these methods are capable of adequately recovering all metabolites of concern.

NOTE: Chemistry Branch I recently concluded that deficiencies in the knowledge of plant metabolism were not applicable for use on sugarcane (PP#2F2765, R. Cook, 11/26/90). We emphasize that this decision applies only to sugarcane and is based on the low total activity (<0.01 ppm) and long pre-harvest interval observed in that crop.

DETAILED CONSIDERATIONS

PLANT METABOLISM

A study on sweet corn was re-submitted by American Cyanamid (1973; MRID 00074621). A discussion of these data is included in the Pendimethalin Residue Chemistry Chapter dated 7/20/84. Greenhouse-grown sweet corn received preemergence soil applications of 4-methyl- or N-ethylpropyl-labeled [^{14}C]pendimethalin at rates equivalent to ca. 1.5 lb ai/A (0.75X the maximum registered rate). Whole plants were sampled 30 and 60 days posttreatment, and grain and cobs were harvested at 81 days posttreatment, and radioassayed for total radioactive residues (TRR). Following methyl-labeled treatments, the TRR in immature plants declined from 0.05 ppm at 30 days posttreatment to <0.02 ppm at 60 days posttreatment; at 81 days posttreatment, the TRR were 0.03 ppm in vegetative parts and 0.02 ppm in grain and cobs. In immature plants (30-60 days posttreatment samples), organosoluble, aqueous-soluble, and unextractable residues accounted for 50-52%, 26-29%, and 19-24% of TRR, respectively; at 81 days posttreatment, 45% of plant TRR were extractable and 55% were unextractable, but no partitioning data were reported. In propyl-label treated plants, the TRR of vegetative parts, grain, and cobs were 0.03 ppm, <0.02 ppm, and <0.01 ppm, respectively. No other data were reported for propyl-labeled treatments. Organosoluble residues partitioned to chloroform or benzene were analyzed by two-dimensional thin-layer chromatography (TLC) in two solvent systems; unaltered pendimethalin (ca. 25-37% of TRR), the 3,5-dinitrobenzyl alcohol metabolite CL 202,347 (ca. 3-4% of TRR), and one unknown (ca. 2-3% of TRR) were identified, but the registrant indicates that the metabolites were not quantified accurately because of a large amount of co-extracted contaminants. Acid and enzyme hydrolyses of aqueous extracts rendered additional ^{14}C -activity chloroform-soluble, but TLC analyses failed to identify any of the residues. Insoluble ^{14}C -residues were not characterized further.

We reiterate our conclusion that this study is inadequate. Polar and insoluble ^{14}C -residues accounting for up to 29% and 55% of TRR, respectively, in both whole plants and grain/cobs, were not characterized. In addition, had the test substance been uniformly ring-labeled, and applied at least 1x the maximum registered rate, sufficient levels of radioactivity may have been available for characterization analyses.

The registrant also re-submitted data pertaining to the metabolism of postemergence-applied, 3,4-methyl-labeled [^{14}C]pendimethalin in forage, silage, cobs, and grain of outdoor-grown sweet corn (1981; MRID 00093698). A discussion of these data is included in the Pendimethalin Residue Chemistry Chapter dated 7/20/84. The applied formulation was modified to contain a

mixture of [^{13}C]- and [^{14}C]pendimethalin to aid in mass spectrometry (MS) identification of metabolites, but MS data were not reported. Outdoor-grown corn received a postemergence treatment at 1.5 lb ai/A (0.75x the maximum registered rate). Plants were sampled at 2, 6, and 12 weeks posttreatment. Residues from 2-week posttreatment plants were radioassayed for TRR, extracted in methanol, and extractable residues were analyzed; 6- and 12-week samples were analyzed for TRR, but the TRR were too low to be further characterized. The TRR decreased from 3.24 ppm in whole plants at 2 weeks posttreatment, to 0.02 ppm in silage at 6 weeks posttreatment, and 0.04 ppm in fodder at 12 weeks posttreatment; TRR in cobs and grain were <0.01 ppm at 12 weeks posttreatment. In plants sampled at 2 weeks posttreatment, methanol-extractable and unextractable residues accounted for 67% and 33% of TRR, respectively; partitioning data were not reported. Two-dimensional TLC analysis of the residues partitioned into dichloromethane isolated components tentatively identified as unaltered pendimethalin (4% of TRR), CL 202,347 (2% of TRR), and the 2,4-dinitrobenzyl alcohol metabolite CL 217,146 (1.3% of TRR); aqueous-soluble and unextractable residues were not fractionated or further characterized.

We reiterate our conclusion that this study is inadequate. Only three metabolites accounting for a total of <8% of the TRR in the 2-week plant sample were identified conclusively. The application rate was insufficient (0.75x), resulting in tissue residue levels too low for characterization in the 6- and 12-week samples.

Data pertaining to the metabolism of [^{14}C]pendimethalin in field and greenhouse-grown peanut hulls were re-submitted by American Cyanamid (1980; EPA ID#GS0187-002). A discussion of these data is included in the Pendimethalin Residue Chemistry Chapter dated 7/20/84. Peanuts were treated with postemergence applications of 4-methyl-labeled [^{14}C]pendimethalin or 3,4-methyl-labeled [^{14}C]pendimethalin, and a pre-emergence application of a [^{13}C]/[^{14}C]pendimethalin mixture, each applied at 0.75 lb ai/A (1x the registered rate for peanuts). Three field studies and two greenhouse studies were conducted. Samples from field, pre-emergence greenhouse, and postemergence greenhouse studies were harvested 129, 159, and 73 days posttreatment, respectively. At harvest, pods were removed from the plants, rinsed in methanol, then the hulls were separated from the seeds and saved for analysis; seeds were discarded. Rinsed hulls were extracted with methanol and acidified methanol, the extracts were combined, and residues were partitioned with chloroform and water. The TRR in peanut hulls from field, pre-emergence greenhouse, and postemergence greenhouse applications were 4.96-5.25 ppm, 1.21-2.48 ppm, and 7.21-8.4 ppm, respectively. Eight metabolites (together comprising 4.5% of the TRR in hulls) were isolated and identified in the initial methanol extracts by two dimensional

TLC (Table 1); at least five additional unknown metabolites (together comprising 28.5% of TRR) also were isolated. Organosoluble and aqueous-soluble residues each accounted for ca. 28% of TRR in one field study; partitioning data for the other field and greenhouse studies were not reported. Metabolites were isolated and identified by two-dimensional TLC using four different solvent systems; many unknowns were isolated, but none were identified. The aqueous phase obtained after adjustment of the hydrolysate to pH 2 was analyzed by HPLC and at least ten unknown metabolites were isolated. Unextractable residues, comprising ca. 18-53% of TRR, were not fractionated or characterized. No data were provided for residues in peanut seeds or foliage.

The peanut study also remains inadequate. Less than 5% of the TRR in peanut hulls was conclusively identified, and no data were provided for peanut seeds and foliage. Insoluble residues accounting for 18-53% of the TRR in hulls were not characterized.

Table 1. Metabolites isolated from peanut hull extracts of peanuts treated with soil-applied, 4-methyl-labeled [¹⁴C]pendimethalin at 0.75 lb ai/A (1x the maximum registered rate for peanuts (data from EPA ID#0187-002)

Metabolite	Code #	% of TRR
pendimethalin, N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzeneamine	CL 92,553	0.9%
4-((ethylpropyl)-amino)-2-methyl-3,5-dinitrobenzyl alcohol	CL 202,347	0.9%
4-((1-ethylpropyl)-amino)-3,5-dinitro- <i>o</i> -toluic acid	CL 99,900	0.3%
3-(2,6-dinitro-3,4-xylidino)-2-pentanol	CL 113,066	0.2%
4-((1-ethyl-2-hydroxypropyl)-amino)-2-methyl-3,5-dinitrobenzyl alcohol	CL 113,067	0.3%
4-((1-ethyl-3-hydroxypropyl)-amino)-2-methyl-3,5-dinitrobenzyl alcohol	CL 113,068	1.3%
4-((1-(carboxymethyl)-propyl)-amino)-3,5-dinitro- <i>o</i> -toluic acid	CL 113,071	0.1%
4-((1-ethyl-2-hydroxypropyl)-amino)-3,5-dinitro- <i>o</i> -toluic acid	CL 113,072	0.5%

Data pertaining to [¹⁴C]pendimethalin metabolism in greenhouse- and field-grown potato tubers was re-submitted by the registrant (1978; MRID 00106795). A discussion of these data is included in the Pendimethalin Residue Chemistry Chapter dated 7/20/84. Greenhouse-grown potatoes were treated with soil-applied, 4-methyl-labeled [¹⁴C]pendimethalin at 2 lb ai/A (1x the maximum registered rate) and harvested for residue analysis at 120 days posttreatment. Field-grown potatoes were treated with a [¹³C]/[¹⁴C]pendimethalin mixture at 1 lb ai/A (0.5x the maximum registered rate) and were harvested at 93-106 days posttreatment. The TRR in greenhouse-grown tubers were 0.13 ppm and 69% was extractable with methanol and acidified methanol. In field-grown

tubers, TRR were 0.08-0.1 ppm and 75-80% of TRR were extractable. No partitioning data were presented. The initial methanol and acidified methanol extracts were combined and analyzed by one-dimensional TLC with one solvent system. Unaltered pendimethalin was tentatively identified but could not be quantified because large amounts of co-extracted substances were present in the extracts; identification was inconclusive due to the lack of confirmatory analyses.

Data requirements for a potato (or other crop with the edible portion grown in the soil such as peanuts) metabolism study remain outstanding. Extractable and unextractable residues accounting for ca. 20-21% and 69-80% of the TRR, respectively, were not adequately characterized. In addition, ring-labeled test substances were not used.

American Cyanamid Company submitted new data (1987; MRID No. 4146 9902) pertaining to the metabolism of pendimethalin in soybean plants following preplant incorporated soil application of 3,4-methyl-labeled- ^{14}C pendimethalin (25.7 $\mu\text{Ci}/\text{mg}$, >99% radiochemical purity). Treatments were applied at 1.5 lb ai/A (0.75x the maximum registered rate). The interval between pesticide application and planting was not specified. Whole plants were sampled at 1, 2, and 4 months posttreatment. Mature plants were separated into straw, hulls, and seeds. Samples were stored frozen prior to analysis, but sample storage intervals were not specified.

Total Radioactive Residues

Samples were analyzed for total radioactivity by combustion/liquid scintillation spectrometry (LSS). Total radioactive residues (TRR) were 0.3 ppm and 0.14 ppm in or on whole plants sampled at 1 and 2 months posttreatment, respectively. At maturity (4 months posttreatment), the TRRs were 0.33 ppm in straw, 0.18 ppm in hulls, and 0.13 ppm in seeds.

Extraction

Samples of whole soybean plants and mature straw were refluxed with 4:1 (v/v) methanol:water for two hours, then homogenized and filtered. The filtrates were concentrated by evaporation and redissolved in 4:1 (v/v) methanol:water. An aliquot of the straw extract was partitioned with hexane, and the aqueous phase was re-partitioned dichloromethane. The organosoluble and aqueous-soluble phases were then analyzed by LSS.

Aliquots from the initial whole plant and straw extracts were refluxed again with 1:1 (v/v) methanol:0.25 N HCl for two hours and filtered. The unextracted solids from 1-month whole plant samples and mature straw samples were refluxed for one hour with

9:1 (v/v) dioxane:0.2 N HCl, then filtered. Insoluble residues were tentatively identified as crude cellulose, but no quantitative supporting data were presented; the filtrates were concentrated by evaporation and diluted with water to precipitate crude lignin, but lignin was not identified conclusively. The putatively extracted cellulose and lignin were radioassayed by combustion/LSS. Unextracted residues were not further characterized. The distribution of the TRR in whole plants sampled at 1-2 months posttreatment and in mature straw sampled at 4 months posttreatment is presented in Table 2.

Soybean seed samples were extracted with 1:1 (v/v) methanol:acetone, homogenized, and filtered. Unextracted residues were re-extracted with 4:1 (v/v) methanol:0.25 N HCl. All extracts and residues were analyzed for total radioactivity. Results are presented in Table 3.

Table 2. Distribution of the total radioactive residue (TRR) in extracts of whole soybean plants and straw following preplant incorporated soil application of [^{14}C]pendimethalin.

Fraction	% of TRR (ppm)		
	1-month plants	2-month plants	straw
<u>Methanol:H₂O (4:1)</u>			
Hexane	- ^a -	- -	2.04 (0.007)
Methylene chloride	- -	- -	4.65 (0.015)
Aqueous			21.13 (0.070)
Total	44.95 (0.135)	51.28 (0.072)	27.82 (0.092)
<u>Methanol:0.25 N HCl (1:1)</u>			
	19.62 (0.059)	12.24 (0.017)	10.22 (0.034)
<u>Unextracted Solids</u>			
<u>Dioxane:0.2 N HCl (9:1)</u>			
Crude Lignin	5.50 (0.016)	- -	6.41 (0.021)
Crude Cellulose	7.08 (0.021)	- -	6.02 (0.020)
	22.85 (0.069)	- -	49.53 (0.163)
Total	35.43 (0.106)	36.48 (0.051)	61.96 (0.204)
Total	100.0 (0.30)	100.0 (0.14)	100.0 (0.33)

^a No data.

Table 3. Distribution of total radioactive residue (TRR) in extracts of soybean seeds following preplant incorporated soil application of [¹⁴C]pendimethalin.

Fraction	% of TRR	(ppm)
Methanol:acetone (1:1)	7.81	(0.010)
Methanol:0.25 N HCl	8.75	(0.011)
Unextracted residue	83.44	(0.109)
Total	100.0	(0.13)

Characterization

Extractable ¹⁴C-residues were first analyzed by HPLC and the following metabolites were tentatively identified by co-chromatography with standards having known retention times: (i) pendimethalin; (ii) its 4-carboxylic acid metabolite (CL 99,900); and (iii) its 3,5-dinitrobenzyl alcohol metabolite (CL 202,347). Pendimethalin, CL 99,900, and CL 202,347 each comprised less than 5% (0.007-0.15 ppm) of TRR. These metabolites represented the only standards used for metabolite identification, even though HPLC analysis of methanol/water extracts from 1-2 month plant samples isolated 25 possible components. None of 25 components contained >10% of the TRR.

One-dimensional TLC analysis, using three different solvent systems and the same three reference standards (having known R_f values), confirmed the presence of pendimethalin, CL 99,900, and CL 202,347 in 1- and 2-month plant samples. In 1-month plant sample extracts, pendimethalin, CL 99,900, CL 202,347 and nine radioactive bands, including a polar band near the origin were identified, but not quantified. In extracts from 2-month posttreatment plants, only trace amounts of CL 99,900 and CL 202,347 were found. Origin-bound residues from the TLC analysis of 1-month posttreatment samples were hydrolyzed with β-glucosidase and re-analyzed by TLC; glucoside conjugates of pendimethalin and its metabolites were tentatively identified, but not quantified.

The distribution of radioactivity into the hexane, methylene chloride, and aqueous phases from the initial mature straw extracts are presented in Table 4. Analysis of aqueous-soluble residues by TLC isolated seven distinct components, but most of the radioactivity remained near the plate origin. An aliquot of the initial methanol/water extract was purified on a Florisil SEP-PAK column, eluted with 40% methanol in ethyl acetate and analyzed by TLC, but only trace amounts of pendimethalin, CL 99,900, and CL 202,347 were isolated.

Table 4. Characterization of the total radioactive residues (TRR) in mature soybean straw 4 months after preplant incorporated soil application of [¹⁴C]pendimethalin at 1.5 lb ai/A (0.75x).

Fraction	% of TRR in straw	(ppm)
Hexane		
Pendimethalin (CL 92,553)	0.22	(0.001)
CL 202,347	0.33	(0.001)
Less polar unknowns (waxy materials)	0.53	(0.002)
Unidentified	<u>0.96</u>	<u>(0.003)</u>
Total	2.04	(0.007)
Methylene Chloride:Methanol		
Most polar unknowns	0.45	(0.002)
Intermediate polar unknowns ^a	3.89	(0.013)
Less polar unknowns	<u>0.31</u>	<u>(0.001)</u>
Total	4.65	(0.016)
Aqueous		
Most polar unknowns ^b	13.24	(0.044)
Intermediate polar unknowns	5.07	(0.017)
Component at RT 71-72	<u>2.82</u>	<u>(0.009)</u>
Total	21.13	(0.070)
Total (in all fractions)	27.82	(0.093)

^a TLC analysis revealed greater than 10 components.

^b TLC analysis revealed 7 bands.

Extractable residues from soybean seed, comprising 16.6% (0.02 ppm) of TRR, were not analyzed by HPLC or TLC. Instead samples of soybean seeds were analyzed for ¹⁴C-residues bound as proteins. Samples were homogenized in 10% sodium chloride solution at 5 C, then centrifuged. The pellet was analyzed by combustion/LSS, and the supernatants (designated by the registrant as protein solutions) were pooled and subjected to a series of ammonium sulfate fractionations; precipitates occurring between 55% and 100% ammonium sulfate saturation were recovered by centrifugation, washed with 95% ethanol, dried, and analyzed for total radioactivity; the results are presented in Table 5. Although 74% (0.096 ppm) of seed TRR was identified as protein on the basis of ammonium sulfate fractionation, no attempts were made to solubilize the insoluble residues enzymatically.

Table 5. Distribution of total radioactive residues (TRR) in soybean seed extracts following preplant incorporated soil application of [¹⁴C]pendimethalin at 1.5 lb ai/A (0.75x).

Fraction	% of TRR in seed	(ppm)
Unextracted unidentified residues	19.7	(0.026)
(NH ₄) ₂ SO ₄ fractionation		
Precipitate (putative protein)	74.0	(0.096)
Aqueous (after precipitation)	0.9	(0.001)
Ethanol wash	5.4	(0.007)
Total	100.0	(0.13)

In summary, these data are insufficient to delineate the metabolism of pendimethalin in soybeans. The low application rate of 0.75x and the failure to use ring labeled material may have precluded identification of many of the isolated components. In addition, only three reference standards were used. The precipitation of insoluble residues in seed using ammonium sulfate is insufficient evidence for characterizing these residues as protein, and hydrolysis of residues should have been attempted. Also, no confirmation was provided for the purported incorporation of ¹⁴C-activity into lignin and cellulose in plants and straw. Finally, none of the residues in soybean hulls, an important cattle feed, were characterized.

REQUIREMENT FOR PROCESSING STUDIES

In the 4/18/90 cover letter American Cyanamid states that no further processing work should be necessary since studies (MRID# 40185102) show that "pendimethalin is not present at the two (2) parts per billion level and thus could not concentrate in a processed commodity to a level above the tolerance value." The registrant is referring to studies for corn grain. With a maximum concentration factor of about 25 to 28, American Cyanamid argues that residues in grain (< 2 ppb) could not concentrate beyond the 0.05 ppm (50 ppb) tolerance (contribution of pendimethalin per se to the 0.1 ppm total tolerance). These data were reviewed previously by Chemistry Branch (aka Dietary Exposure Branch, D. Edwards, 9/8/89) and concluded that the tolerance for grain should be lowered to 2 ppb and a processing study conducted using corn grain treated at the highest practical exaggerated rate.

With regard to the need for processing studies, the Agency has reconsidered the situation for corn and concluded that a corn processing study is not required, provided additional residues of concern are not found in the additional plant metabolism studies. However, in the absence of a processing study for corn, processing studies for all other oilseed crops on which pendimethalin is registered are required per the current Chemistry Branch policy. The pertinent crops should be treated in the field at the highest practical exaggerated rate and then processed into those fractions cited in the Residue Chemistry Guidelines. If any particular crop can be treated at rates higher than the maximum theoretical concentration factor and no detectable residues of concern are found in the seed, the processing study may be waived for that crop. Chemistry Branch emphasizes that the intentional setting of a tolerance on a raw agricultural commodity (e.g., corn grain) high enough such that residues in a processed commodity (e.g., corn oil) will not exceed that tolerance will not be accepted in the future.

July 25, 1989

MEMORANDUM

SUBJECT: Guidance on When and How to Conduct Livestock Metabolism Studies.

FROM: Richard D. Schmitt, PhD, Chief
Dietary Exposure Branch
Health Effects Division (H7509C)

TO: Dietary Exposure Branch Staff

This memo is intended to clarify when livestock metabolism studies are needed and the efforts that should be made to identify residues in such studies. The emphasis will be on those cases where low or non-detectable residues are observed in feed items. The need for conventional livestock feeding studies in such situations will also be addressed.

For many years Branch policy called for livestock metabolism studies only when detectable residues of concern were found in feed items in crop field trials. This procedure discriminates against registrants who develop more sensitive methods for measuring residues in crops. In recent times we have requested animal metabolism studies in some instances when no detectable residues are present in feed items. However, this procedure has not been consistently applied. Effective with this memorandum, it will be Branch policy to require livestock (ruminant and/or poultry) metabolism studies whenever a pesticide is to be applied to a crop having an animal feed listed in Table II of the Residue Chemistry Guidelines. In addition to not discouraging the development of more sensitive methods for crop residues, this policy will provide some information on the potential transfer of residues to meat and milk in those cases where misuse results in residues in feed items not expected to have residues from approved uses.

Since ruminant and poultry metabolism studies are now to be required in cases where very low or non-detectable residues are present on feed items, the question arises as to what dose level is appropriate. Following FAO's lead, we suggest ≈ 10 ppm in the diet as the dosing level. This will typically represent 200-1000x the anticipated dietary burden for livestock when trace residues occur in feed items. The radiolabeled material should have sufficient specific activity to enable detection of ≈ 0.01 ppm (10 ppb) in milk, tissues, and eggs.

As already stated in the Guidelines, the ruminants and/or poultry (depending on feed items involved) should be dosed with radiolabeled material for at least three consecutive days with milk or eggs collected daily. Animals should be sacrificed within 24 hours of the final dose and total radioactivity measured in edible tissues and milk/eggs.

With regard to the degree of characterization of residues in animal (and plant) metabolism studies, a strategy developed by Ciba Geigy may be used as a guide to what effort should be expended (Reference 1). If total activity in a tissue (or crop part) is ≈ 0.01 ppm (10 ppb) or less, no differentiation of the radioactivity would be required. For activity greater than ≈ 0.01 ppm the sample should be extracted with organic solvents (perhaps mixed with neutral water). The levels of extractable

and non-extractable (NE) activity should then be quantitated to determine the degree of characterization that is needed. If the extractable activity represents ≈ 0.01 ppm or less in the original sample, it need not be examined further. For extractable activity of ≈ 0.01 - 0.05 ppm, the partitioning behavior between aqueous and an organic solvent should be determined followed by chromatographic (TLC, HPLC) analysis of the organosoluble activity. The chromatographic behavior of this activity can be compared to that of the parent pesticide and likely metabolites. When the extractable activity exceeds ≈ 0.05 ppm, complete characterization should usually be attempted (however, note discussion on highly exaggerated doses below). Identities of metabolites should be confirmed with a second technique, spectroscopic if possible.

With regard to the non-extractable (NE) residues obtained above, further characterization should be attempted when the NE activity in a sample is greater than ≈ 0.1 ppm or 10% of the total activity in that sample. Attempts should be made to release these residues by acidic or basic hydrolyses and/or enzyme treatments. The degree of characterization needed for the released activity will depend upon its total level in ppm using the figures outlined in the previous paragraph (i.e., ≈ 0.01 , 0.01 - 0.05 , and >0.05 ppm) for extractable residues.

It should be noted that the above ppm "trigger" values are not absolute requirements, but rough guides as to how much characterization is adequate. In the metabolism studies wherein highly exaggerated feeding levels are employed and low activity results in tissues, characterization requirements should be less stringent than when expected dietary burdens lead to significant activity in animal products. For example, if the anticipated dietary burden to livestock is about 0.01 ppm, 10 ppm radio-labeled compound is fed ($1000\times$), and total activities in tissues, milk or eggs are ≤ 0.1 ppm, minimal characterization of residues should be adequate (unless toxicologists express a special concern with residues at this level). Such situations often arise with early season herbicides having low application rates.

On the other hand, when activities ≥ 0.1 ppm are observed in animal commodities from ingestion of the pesticide at levels expected on feed items, thorough identification of the residues is generally required. This is likely when pesticides are applied foliarly at high rates through the entire growing season.

Finally, with respect to the need for conventional feeding studies, such data will not be required when no detectable residues are observed in feed items from crop field trials reflecting the proposed use of the pesticide (maximum rate, minimum preharvest interval) unless the metabolism study shows potential for significant bioaccumulation. When trace residues are detected in the field trials, the reviewer should consider the anticipated dietary burdens and the results of the radiolabeled metabolism study when determining whether feeding studies are necessary. In the previously cited example (0.01 ppm dietary burden, $1000\times$ dose leading to <0.1 ppm total activity in meat/milk/eggs), a feeding study would not be necessary as expected residues in animal commodities from ingestion of 0.01 ppm would be on the order of ≈ 0.1 ppb (assuming linear relationship between dose and residues). In this case the metabolism study also serves as a feeding study and tolerances would not be needed for meat, milk, poultry and eggs.

SUMMARY

•Livestock (ruminant and/or poultry) metabolism studies will be required whenever a pesticide is to be used on a crop having a livestock feed item in Table II of the Residue Chemistry Guidelines.

- "Trigger values" (ppm radioactivity) are provided as rough guides to the degree of characterization needed in metabolism studies. However, reviewers should take into account how the rate or dose used in the metabolism study compares to that expected from actual use of the pesticide.
- When considering the need for conventional feeding studies in cases where low residues are found in feed items, reviewers should take into account the expected dietary burdens and results of animal metabolism studies. The latter may indicate that residues in animal commodities would be well below detection limits and thus serve as feeding studies. Meat/milk/egg tolerances would not be necessary in such situations.

Reference 1. "STRATEGY FOR DETERMINATION OF EXTENT OF METABOLISM STUDIES AND DEVELOPMENT OF RESIDUE METHODS BASED ON TRIGGER VALUES", 1/27/88, Dr. B. Donzel, Ciba-Geigy.

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