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

APR 17 1991

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP#9F3770 -- Lambdacyhalothrin for Dermal Application to Beef Cattle. Response to CBTS 1/25/90 memo. Submission Dated 10/12/90.

DEB #'s 7222, 7223, 7768. No MRID #.

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Coopers Animal Health, Inc. has proposed the following tolerances for lambdacyhalothrin, RS-alpha-cyano-3-phenoxybenzyl (1RS)-cis-3-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate:

Cattle fat	1.0 ppm
Cattle meat and meat byproducts	0.1 ppm

The present submission is a response to our 1/25/90 memo.

Summary of Deficiencies Remaining to Be Resolved

--- Label must be revised to limit number of applications.

--- Label must contain restriction against application to dairy cattle.

--- EPA method validation and multiresidue protocol testing necessary for any metabolite that is regulated.

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--- Residue data necessary for HO-CPA unless it is identified as a rat metabolite.

Conclusions

1a. Because no residue data are available which show that dermal application of Lambdacyhalothrin plateau after a certain number of applications at two week intervals, the petitioner should submit a revised Section B in which the maximum number of applications is limited to 4 within any six month period. Alternatively, additional residue data may be submitted to show that a plateau is eventually reached.

1b. Because residues present in a non-lactating pregnant cow would carry over into milk when the cow becomes lactating, the petitioner should submit a revised Section B containing the restriction "Do not apply to dairy cattle." [The petitioner intends to submit such a revision.]

2a. The nature of the residue in fat, muscle and milk of cattle is adequately understood. Lambdacyhalothrin is the only major component of the residue.

2b. The nature of the residue in liver and kidney is now adequately understood. In addition to parent, the following metabolites are present in significant quantities: CPA [(1RS)-cis-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane-carboxylic acid], free and conjugated; HO-CPA [(1RS)-cis-3-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2-hydroxymethyl-2-methylcyclopropanecarboxylic acid], free and conjugated; 3PBA [3-phenoxybenzoic acid], free and conjugated; and 4'OH-3PBA [3-(4'hydroxy)-phenoxybenzoic acid], free and conjugated.

CBTS and TB1 have determined that sufficient information is available on CPA, 3PBA and 4'OH-3PBA to permit the conclusion that these species need not appear in the tolerance expression. However, unless tha registrant can demonstrate that HO-CPA is a metabolite of lambdacyhalothrin in the rat, residue data must be submitted before a similar conclusion can be drawn. The data must be supported by a validated analytical method.

3. The submitted analytical methods are acceptable. However the analytical methods for those metabolites which appear in the tolerance expression must undergo independent laboratory validation and then EPA method validation. No method was submitted for metabolite HO-

CPA. If EPA determines that this metabolite must be regulated (see previous conclusion), the petitioner must develop a method for this metabolite. Recoveries must be obtained under FDA's multiresidue protocols for the regulated metabolites.

4. Residue data support the proposed tolerances. However, data for HO-CPA were not submitted. Unless the registrant can demonstrate that this metabolite is also a rat metabolite, residue data will be necessary.

Recommendations

CBTS recommends against the proposed tolerances for reasons given in Conclusions 1a and 1b (revised Section B necessary), Conclusion 2b (question concerning metabolite HO-CPA), Conclusion 3 (analytical method for HO-CPA, if necessary), and Conclusion 4 (residue data for HO-CPA, if necessary).

Detailed Considerations

Deficiencies as listed in our 1/25/90 memo are listed followed by the registrant's responses and CBTS' comments.

Deficiency #1a (Conclusion #1a of our 1/25/90 memo)

The proposed label directions set no limit on the number of applications. In the absence of data which show that residue levels plateau when lambdacyhalothrin is applied at two week intervals, the petitioner should submit a revised Section B in which the maximum number of applications is limited to 4 within any six month period.

Petitioner's Response

No data are available which show definitively that residue levels have reached a plateau following four treatments at intervals of 14 days. However, it was the registrant's intention that the residue study in the protocol would support full season use at intervals of 14 days. The protocol was submitted to EPA for comment and approved by the Residue Chemistry Branch on 12/8/87.

CBTS Comment

As noted in our previous memo, we have no data which indicate that residue levels plateau. The dermal residue study is acceptable, but in the absence of such data there must be a label restriction concerning the number of applications. The petitioner may wish to generate additional residue data to show that residues plateau after a certain number of applications. This deficiency remains.

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CBTS Deficiency #1b

The proposed label restricts use of lambdacyhalothrin to beef cattle, non-lactating dairy cattle and calves. However, because residues present in a non-lactating pregnant cow would carry over into milk when the cow becomes lactating, the petitioner should submit a revised Section B containing the restriction "Do not apply to dairy cattle".

Petitioner's Response

Section B will be amended as requested.

CBTS Comment

Once such as amendment is received, this deficiency will be resolved.

CBTS Deficiency #2c

The nature of the residue in liver and kidney of cattle treated dermally with lambdacyhalothrin is not understood....

Acid Labeled Residue -- Liver

Only 32.6% of the acid labeled residue in liver was identified. The polar unknown constituting 13.2% of the total radioactive residue (TRR) as determined by TLC using solvent system E should be isolated and subjected to acid hydrolysis to demonstrate that it is indeed a conjugate of HO-CPA....The three identified components -- parent, CPA..., and HO-CPA should be separated from the other components using TLC and HPLC and these other components should be acid hydrolyzed. Any species present at ≥ 10% of TRR should be identified.

Petitioner's Response

The petitioner has responded as follows:

We agree that not all metabolites have been identified but we do not agree that any single metabolite is present in this unassigned residue which constitute more than 10% of the Total Radioactive Residue (TRR)....apart from the 15.6% which was not extracted following hot 6M acid treatment and 11.7% which was not extracted from the hydrolysis solution no unknown metabolite exceeded 10% of TRR.....The extraction or characterisation of material which remains in aqueous solution would not be improved if the known components were first removed as suggested. The experiment was conducted as described not because we wanted to identify any particular minor component but to obtain the total concentration of CPA

and HO-CPA in the tissue (free + conjugates).

We cannot find ... a single unknown which constitutes 13.2% of TRR..... The only values which give results close to 13.2% would be taken directly from AMBIS scans [results of which] are as a percentage of the extract not as a percentage of TRR.....

CBTS Comments

A re-review of the original data shows that the unknown reported in our memo as present at 13.2% of TRR was present at 13.2% of the acetonitrile extract, or 7.0% of TRR. The petitioner is correct on this point. We have carefully examined chromatograms from the acetonitrile extracts and corresponding chromatograms from the various organic extracts from acid extracts in order to see if common unknown moieties exist which in sum might be greater than 10% of TRR. There do not seem to be such common moieties. We agree that a residue unextractable after refluxing with 6M HCl is probably irreversibly bound and not of concern.

As reported in our earlier memo, the petitioner acidified the organic (acetonitrile) extract to 2M HCl and heated the extract at 80°C for 4 hours. The petitioner states above that this was done not to identify any minor component but to determine the total concentration of CPA and HO-CPA (free and conjugate) in the tissue. However, as noted in our memo, parent lambdacyhalothrin was apparently hydrolyzed by this procedure and should have been removed from the extract before hydrolysis. A slight increase in the sum of parent, CPA and HO-CPA was observed after hydrolysis, presumably due to hydrolysis of conjugates. The qualitative nature of the residue remains unchanged. As noted in our previous memo, the unknown from solvent system E (reported as 13.2% of TRR) was absent in the hydrolyzed extract and could have been converted into HO-CPA if it were a conjugate of that species. Because the unknown constitutes only 7% of TRR, we now see no need to isolate the unknown compound and subject it to acid hydrolysis to see if it hydrolyzes to HO-CPA, as was requested in our memo.

CBTS concludes that the nature of the acid labeled residue in liver has been sufficiently characterized, even though only about one-third of the residue has been identified.

CBTS Deficiency #2c (cont.)

Alcohol Labeled Residue -- Liver

47.4% of the alcohol labeled residue in liver was identified. The principal constituent was "L3", apparently

a conjugate of 3PBA. The petitioner should provide additional evidence for the identity of this compound. This can be done using mass spectrometry but, alternatively, through chromatography using different solvent systems.

Petitioner's Response

The petitioner agrees that further work to characterize L3 is necessary. This has been done (report CIBH 89-2) identifying this metabolite by mass spectrometry as a glutamic acid conjugate of 3PBA. The report also gives further information on metabolites L1 and L2. The report is dated 6/16/89.

The mass spectrum of the methylated metabolite L3 is consistent with the above identification as a glutamic acid conjugate of 3PBA. An attempt to synthesize the cold standard was unsuccessful, however, so we do not have a mass spectrum of a standard for comparison. The apparent molecular ion (m/z 371) is that of the diester of the glutamic acid conjugate, and the intense component m/z 197 corresponds to the phenoxybenzoyl fragment ion of 3PBA. Association of L1 with 3PBA by mass spectrometry is more tentative, and no obvious association exists with L2.

CBTS Comment

Although the registrant has not conclusively proved that metabolite L3 is the glutamic acid conjugate of 3PBA, we are satisfied that L3 is a conjugate of 3PBA. At this time we have no further questions. This deficiency is resolved.

CBTS Deficiency #2c (cont.)

Kidney

About 58% of the acid labeled residue in kidney was identified. Lambdacyhalothrin was the major component (38% of TRR) followed by CPA (15.5%) and HO-CPA (4.0%). However, HPLC of extracts from the alcohol labeled residue showed lambdacyhalothrin as only 19.8% of TRR; and TLC of extracts showed no parent at all. The principal component of the residue as determined by TLC was "K3", apparently a conjugate of 3PBA, present at up to 46% of TRR. The petitioner should explain the difference in parent concentration found in acid labeled and alcohol labeled residue as well as the different results obtained with TLC and HPLC on the same alcohol labeled residue.

Petitioner's Response

Parent is not expected to be present at the same percentage of TRR with the different labels. The acid label TRR is the

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concentration of parent plus acid metabolites and the alcohol label TRR is the concentration of parent plus alcohol metabolites. The alcohol and acid metabolites will clear from tissue at different rates. TRR in kidney from the acid label is 0.065 and 0.142 ppm equivalents; that from the alcohol label is 0.1165 and 0.1323 ppm equivalents. When the percentages obtained from HPLC are applied to these ppm equivalents, ppm parent in the kidney samples from cows treated with the acid label are 0.025 and 0.054 ppm; corresponding concentrations in kidney samples from cows treated with the alcohol label are 0.023 and 0.026 ppm.

The petitioner believes that the different results obtained from TLC and HPLC are due to the much lower amounts examined by TLC (1000 dpm vs 42,500 and 17,000 dpm). The parent, having the greatest Rf, would be the most diffuse spot on the TLC plate. In the study with acid labeled lambdacyhalothrin, considerably more activity was applied to the TLC plate. Percentages agreed from TLC's taken in differing solvent systems. (HPLC was not used for the acid label.) Analyses of kidney samples by the validated residue method showed residues of 0.019 and 0.030 ppm, in good agreement with the HPLC results.

CBTS Comment

The registrant has satisfactorily responded to our questions. This part of Deficiency #2c is resolved.

CBTS Deficiency #3c

Analytical methods have been submitted only for metabolites CPA, 3PBA and 4'OH-3PBA. Satisfactory recoveries were obtained using these three methods, but from results from the metabolism studies it is not clear that the method for 4'OH-3PBA will distinguish that metabolite from a conjugate of 3PBA. The petitioner should comment on the possibility that the analytical method for 4'OH-3PBA is not specific for this compound.

Petitioner's Response

The residue method uses a different chromatography system from that employed in the metabolism study, which shows an interfering peak coincident with 3PBA. The residue analytical method contains a deconjugation step which would hydrolyze the conjugate to 3PBA. It was not really possible to compare relative amounts of metabolites found in the labeled experiment with results of residue analysis of the same tissue because such residues were close to the limit of reliable measurement.

CBTS Comment

The extraction procedure in the metabolism study (alcohol 7

label) involved sequential solvent extraction using hexane, ether, acetonitrile, acetonitrile:0.1M HCl, 0.1M HCl and 0.1M NaOH. Unknown L3, which co-chromatographed with 4'OH-3PBA, was shown to be a conjugate of 3PBA. In attempts to identify L3 (pp. 13-14 of our earlier memo), the unknown was hydrolyzed by heating in 4M HCl at 75°C for three hours. The residue analytical method for conjugates, discussed on p. 18 of our memo, contains a deconjugation step in which the extract is heated in 4M HCl at 75°C for four hours. Therefore, it is likely that the residue analytical method would deconjugate L3 and remove the potential interference. This part of the deficiency is resolved.

CBTS Deficiency #3c (cont.)

No method was submitted for HO-CPA. Once outstanding metabolism data have been submitted, the HED Metabolism Committee will be asked to determine whether regulation of metabolites is necessary. If regulation is necessary a method for this metabolite will have to be developed, the methods for the metabolites will have to undergo EPA method validation, and the specificity of the method for 4'OH-3PBA may have to be resolved.

Petitioner's Response

No method was available for HO-CPA and no method was successfully developed for this metabolite at the time of the work. An earlier study (YIBH 85-C12) with orally dosed animals had shown that this metabolite was less significant than CPA, and this was confirmed in the dermal metabolism study. The petitioner believes that the determination of residues of CPA can be used to indicate the residues of HO-CPA. It is also noted that concentrations reported from the metabolism study are in "ppm equivalents", i.e., determined assuming the same molecular weight as lambdacyhalothrin, 449. If concentrations are calculated using the molecular weight of HO-CPA, 258, the 0.03 ppm equivalents total concentration of HO-CPA found in liver would be reduced to 0.017 ppm. The corresponding concentration for CPA would go from 0.04 ppm to 0.022 ppm. These concentrations resulted from treatment at the maximum recommended volume for three successive days. Residue studies carried out at the recommended label rate -- one treatment every 14 days -- would produce HO-CPA levels below the limit of reliable measurement (0.01 ppm) as was found for residues of CPA.

CBTS Comment

We agree that concentrations of metabolites will be low. A 4/11/91 meeting with TB1 dealt with the issue of which, if any, of the various metabolites of lambdacyhalothrin should appear in the tolerance expression. CPA, 3PBA and 4'OH-3PBA are products of rat metabolism and are found in tissue at only low levels. It

was decided that these metabolites need not appear in the tolerance expression. However, HO-CPA was not identified as a rat metabolite, and no residue data are available. Metabolism data suggest that levels of HO-CPA will be lower than corresponding levels of CPA (see our memo of 1/25/90); but unless the registrant can demonstrate that HO-CPA is a rat metabolite, residue data on HO-CPA -- and a validated analytical method -- will be needed before a final decision can be made.

CBTS Deficiency #4b

> Residue data...support the proposed tolerances...However data for HO-CPA were not submitted, and it is not clear that the analytical method for 4'OH-3PBA is specific for this metabolite. Depending on the conclusions of the HED Metabolism Committee, these deficiencies may have to be addressed.
>
> See our previous comments.

Attachment: Deferral memo to TB.

cc: SF, RF, Circu., C.Furlow(PIB/FOD), MikeFlood, E.Haeberer, PP#9F3770.

H7509C:CBTS:Reviewer(MTF):CM#2:Rm800A:557-4362:typist(mtf):4/17/91.
RDI:SectionHead:ETHaeberer:4/4/91:BranchSeniorScientist:RALoranger: 4/4/91, 4/12/91.

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