

PMSD/ISB



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

**EXPEDITE**

AUG 03 1989

OFFICE OF  
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP#6F3392/6H5500; 9F3705/9H5572. Clofentezine on Apples. Amendment of May 10, 1989.  
MRID Nos. 410930-00 thru -07  
DEB Nos. 5367, 5368, 5369, 5370, 5371  
HED No. 9-1405A.

FROM: Martha J. Bradley, Chemist *M J Bradley*  
Dietary Exposure Branch  
Health Effects Division (H7509C)

TO: Dennis Edwards, PM 12  
Insecticide-Rodenticide Branch,  
Registration Division (H7505C)

and

Toxicology Branch, IRS  
Health Effects Division (H7509C)

THRU: Richard D. Schmitt, Ph.D., Chief *Richard D Schmitt*  
Dietary Exposure Branch  
Health Effects Division (H7509C)

This review has been expedited at the request of Anne E. Lindsay, Director of Registration Division, memo of June 8, 1989. The expedited due date is August 6, 1989.

Nor-Am Chemical Company has submitted an amendment in response to the deficiencies listed in our (F. Griffith) reviews of February 28 and March 8, 1989 in connection with these two petitions.

The major deficiencies are the same for the two petitions and since only the tolerances for the use likely to lead to the greater residue will be recommended, the deficiencies for the two petitions will be combined and discussed below.

**Summary of the Chemistry Deficiencies Remaining to be Resolved**

A revised Section F for the tolerance proposal.

Completion of successful method trials

**Conclusions**

1. The nature of the residue in ruminants has been adequately delineated. The residue of concern is clofentezine and its 4-hydroxyclofentezine metabolite.
2. A method trial has been requested for the three new methods for clofentezine and 4-hydroxyclofentezine in animal tissues and milk. Resolution of the final analytical methodology deficiency depends on a successful method trial.
3. The tolerance proposals for the animal tissues should read for meat, fat and meat by-products, except liver, of cattle, goats, hogs, horses and sheep at 0.05 ppm and for liver of cattle, goats, hogs, horses and sheep at 0.4 ppm.

**Recommendations**

We recommend against the proposed tolerances because of Conclusions 2 and 3. Favorable consideration depends on a successful method trial and a revised tolerance proposal for animal tissues.

**Detailed Considerations****Deficiencies Related to Directions for Use**

1. The petitioner should revise the label to prohibit aerial application or specify ground application only.

**Petitioner's Response**

1. The directions for use now read "Do not apply by air".

**Comments/Conclusions**

This deficiency has been resolved.

### **Deficiencies Related to Ruminant Metabolism - Nature of the Residue in Livestock**

1. The petitioner should account for the qualitative difference in metabolites in milk from the cattle and goat studies.
2. Additional identification of the residue in bovine liver, kidney and fat is needed.
3. Additional information is needed for the in-life portion of the bovine metabolism study as well as details of the determination of total radioactivity of the tissues in this study.

### **Petitioner's Response**

1. The tentatively identified 3- and/or 6-methoxy clofentezine found in the methylated residue in goat milk is attributed to incomplete dissociation of clofentezine metabolites with endogenous material.

**MRID 410930-06 M53 Clofentezine: Comparison and Characterisation of [14C]-residues in milk of the cow and goat following oral dosing with [14C]-clofentezine (2.2 mg/kg/day). ENVIR/89/15 by N.W.A. Phillips and L. Swalwell. Study No.: 94J.**

Milk from a previous goat study, Registration reference M47, was reanalyzed using the same methods as used on the milk in the cow study, Registration reference M52, including the use of snail digestive enzymes. The goat milk samples also gave a single peak on tlc co-chromatographing with 4-hydroxy clofentezine. Since the goat milk had been stored for about 2 years, a further goat was dosed with <sup>14</sup>Cclofentezine at the same rate, 2.2 mg/kg/day for 3 days, and fresh milk samples were obtained for analysis.

Details of the in life study and total radioactivity counting procedure for the new goat feeding study are submitted. Total activity was 0.21 microgram equivalents/ml, similar to that in the second goat study of 0.24 microgram equivalents/ml. Cow and goat milk samples, both stored and fresh, were extracted with methanol, the extract treated with snail juice enzymes, cleaned up on LC Diol cartridges and analyzed by tlc by any of 4 systems against 3-, 4-, 5-, and 6-OH clofentezine, 3-OH 2-SMe clofentezine and parent compound. A sole major peak of activity co-chromatographing with 4-OH clofentezine was present in extracts from both species. The extracts, when methylated and chromatographed against 3-, 4-, 5-, and 6-methoxy clofentezine showed a single peak co-chromatographing with 4-methoxy clofentezine in cow milk and was a major peak in goat milk. However, more polar peaks were visible in the goat milk. On further cleanup including preparative tlc to remove co-extractives which interfered with the methylation procedure, only a single major component co-

chromatographed with 4-methoxy clofentezine.

2. **MRID 410930-03 M54 Clofentezine:** Characterisation of the [14C]-residues in tissues of a cow following oral administration of [14C]-clofentezine (2.2 mg kg<sup>-1</sup> day<sup>-1</sup>) for 3 days ENVIR/89/19 by N.W.A. Phillips and L.M. Swalwell. Study No.: 94J.

Samples of liver, kidney, renal fat and urine, collected from the cow study 92J (Registration reference M52) and stored at -20°C for ca 4 months, were utilized to further identify the residue. The petitioner states that data produced during the study has not shown that any breakdown or degradation of the samples occurred during this storage period. (A storage stability study reviewed by A. Smith, 12/5/87, PP7F3511/7H5535, showed that the parent compound in tissues and milk degraded after 6 months of storage but the total residue, determined by conversion of metabolites to orthochlorobenzoic acid, was essentially recoverable for up to 15 months in frozen storage.)

Total activity in liver, kidney and fat was 0.76, 0.357 and 0.262 ppm. Free or unbound 4-OH clofentezine comprised 67, 83, and 90% of the liver, kidney and fat residue. The bound residue in liver, kidney were extensively investigated using collagenase, subtilisin and snail juice enzymes, acid and base hydrolysis cleanup by LC Diol C18 Bond Elut and Silica cartridges, and ethyl acetate/hexane/acetonitrile extractions. Small amounts of 4-OH clofentezine were identified as well as products with similar chromatographic properties to spectroscopically identified hydrolytic breakdown products of 4-OH clofentezine. It is apparent that in order to release the bound metabolites, procedures have to be used that result in the further degradation of the residue.

3. The additional information requested for the in-life portion and total radioactivity determination of the bovine metabolism study is submitted.

#### **Comments/Conclusions**

1. The nature of the residue in milk has been adequately delineated. The residue consists primarily of the metabolite 4-hydroxy clofentezine.

This deficiency has been resolved.

2. The nature of the residue in animal tissues has been adequately delineated. The residue consists primarily of the metabolite 4-hydroxy clofentezine.

This deficiency has been resolved.

3. This deficiency has been resolved.

#### **Deficiencies Related to the Residue Analytical Method**

1. Depending on the resolution of the ruminant metabolism questions, analytical standards for clofentezine metabolites, in addition to that for 4-hydroxy clofentezine which has been submitted, may be needed.
2. If the petitioner wishes the "total residue" method for milk to be the confirmatory method, the 1.6 correction factor should be clarified.
3. The new HPLC procedure for clofentezine, per se, should be combined with the previously submitted clofentezine metabolites method for meat and milk.
4. Additional validation data is needed for the hydroxy clofentezine metabolites to be tolerated as well as for the parent compound.
5. The U.S. grade of snail digestive juice needs to be clearly defined.

#### **Petitioner's Response**

**MRID 410930-01 S50 Clofentezine:** Response to 1989 EPA DEB Reviews of clofentezine meat and milk residue/metabolism data.

1. The new ruminant metabolism data show there is no need for analytical standards for other clofentezine metabolite standards.
2. The "total residue" method is no longer proposed as a confirmatory method.
3. Due to the difference in polarity of clofentezine and hydroxy-clofentezine, different extraction techniques, cleanup steps, elution solvent strengths and different absorption maxima are needed. It is unlikely that a combined method would save time compared with performing two individual methods.
4. Three new methods are submitted: one for 4-OH clofentezine in milk and fat meat; one for clofentezine in milk and fat meat; one for clofentezine and 4-OH clofentezine in animal tissues other than fat.

**MIRD 410930-05 R182 (2nd edition) Clofentezine:** Analytical method for the determination of residues of 4-hydroxyclofentezine

in milk and animal fat by high performance liquid chromatography  
RESID/89/49

Residues of 4-hydroxyclofentezine are extracted from milk by ultrasonic agitation with acetone and from fat samples by reflux in hexane/acetonitrile. Milk residues are enzymatically hydrolyzed with snail digestive juice and cleaned up by partition into ethyl acetate/hexane. Fat extracts are cleaned up by elution through a Supelclean diol-phase cartridge. Residues of 4-hydroxyclofentezine are determined by normal phase HPLC with detection by monitoring UV absorption at 301 nm. Residues may be confirmed by reverse-phase conditions.

Analysis of a batch of eight samples takes approximately one day to the HPLC determination which takes a further 2 hours. The limit of determination is estimated as 0.01 ppm for milk and 0.05 ppm for fat. Specifications are given for the analytical reagents including a USA source for the snail juice enzymes. Recoveries from fortification levels in milk of 0.01 to 0.2 ppm range from 70 to 103%. From fortification levels in fat of 0.07 and 0.14 ppm, recoveries range from 67 to 104%. Control samples for fat were 0.002 to 0.005 ppm and for milk were up to 0.005 ppm. Recoveries by reverse phase HPLC were comparable to those by normal phase HPLC.

The method was used to analyze samples from the radiolabeled cow study. Mean recoveries of 83 and 86% were obtained from milk and fat, respectively.

**MRID 410930-02 R54 (3rd edn) Clofentezine: Analytical Method for the Determination of Free Clofentezine Residues in Milk & Animal Fat by High Performance Liquid Chromatography**  
RESID/89/50

Minced fat samples are homogenized with dichloromethane and methanol to extract clofentezine. Milk is extracted by shaking with hexane and diethyl ether after breaking the milk fat globule membrane with potassium oxalate/ethanol. The extracts are concentrated and cleaned up by acetonitrile/hexane partition to remove fat, followed by elution through a Sep-pak silica cartridge. Final determination is by normal phase HPLC, with detection by clofentezine by monitoring UV absorbance at 268 nm. Confirmation of residues is by reverse phase HPLC.

Analysis of a batch of six samples takes approximately one day to the HPLC determination which takes a further one and one half hours. The limit of determination is estimated as 0.01 ppm for milk and fat. Specifications are given for the analytical reagents. Recoveries from fortification levels in milk of 0.01 to 0.2 ppm range from 74 to 99%. From the same fortification levels in fat, recoveries range from 64 to 92%. Control samples

for fat were 0.005 to 0.02 ppm and for milk were up to 0.001 ppm. Recoveries by reverse phase HPLC were comparable to those by normal phase HPLC.

**MRID 410930-04 R72 (3rd edn) Clofentezine: Analytical method for the determination of residues of clofentezine and 4-hydroxy clofentezine in animal tissues by gas chromatography RESID/89/51**

Tissue samples are hydrolysed with hydrobromic acid to convert 4-hydroxyclofentezine to 2-chlorobenzoic acid (2-CBA). After partition into diethyl ether followed by back partition between alkali and ether, extracts are concentrated and 2-CBA residues are methylated with diazomethane to facilitate final determination by gas chromatography with mass selective detection using selected ion monitoring.

Analysis of a batch of six samples takes approximately one day to the GC/MSD determination which takes approximately seven hours. The limit of determination is estimated as 0.05 ppm for all tissues. Specifications are given for the analytical reagents. Recoveries from 4-hydroxyclofentezine fortification levels in liver, kidney and muscle tissue of 0.025 to 0.25 ppm range from 67 to 102% with a mean of 87%. Recoveries from clofentezine fortification levels in liver, kidney and muscle tissue of 0.1 and 0.25 ppm range from 74 to 127% with a mean of 97%. Control samples for liver, muscle and kidney were 0.003 to 0.008 ppm. Confirmatory information is provided from ratios of selected ions.

The method was validated using aliquots of liver and kidney from the cow <sup>14</sup>C study. The extractable residue was accounted for to the extent of 86% in liver and 83% in kidney.

#### **Comments/Conclusions**

1. This deficiency has been resolved.
2. This deficiency has been resolved.
3. This deficiency has been resolved.
4. A method trial has been requested for the three new methods for clofentezine and 4-hydroxyclofentezine in animal tissues and milk. Resolution of the final analytical methodology deficiency depends on a successful method trial.
5. This deficiency has been resolved.

Clofentezine dietary exposure review

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**Comments/Conclusions**

The tolerance proposals for the animal tissues should read for meat, fat and meat by-products, except liver, of cattle, goats, hogs, horses and sheep at 0.05 ppm and for liver of cattle, goats, hogs, horses and sheep at 0.4 ppm.

cc: M. Bradley, RF, Circu, PP6F3392, PP9F3705, PMSD/ISB  
H7509C:DEB:M Bradley:mb:CM#2:Rm810:557-7324:07/06/89  
RDI:RSquick:07/28/89:RDSchmitt:07/31/89