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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SEP 30 1993

MEMORANDUM

Subject: Reregistration of Mepiquat Chloride (List B, Case 2375, Chemical 109101). BASF Corporation Nature of the Residue in Ruminants and Poultry Submissions. CBRS Nos. 10685 and 11386. DP Barcodes D183217 and D188232. MRID Nos. 42394301 - 42394304 and 41585201 - 41585204.

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Attached is the review of BASF Corporation's response to the Phase 4 Review. The residue chemistry issues addressed in this submission are nature of the residue in poultry, nature of the residue in ruminants, and radiovalidation of the analytical method. This information was reviewed by Acurex Environmental Corporation under supervision of CBRS, HED. The data assessment has undergone secondary review in the Branch and has been revised to reflect Branch policies.

The nature of the residue study in poultry is fully acceptable. The residue of concern is the parent, mepiquat chloride.

The nature of the residue in ruminants study is not acceptable, but may be upgraded by the submission of additional information. Specifically, the in-life and storage information for the samples generated for the radiolabled validation (1991; MRID 42394303), and

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analyzed for metabolism purposes (1991; MRID 42394301) is needed. The required information includes: the specific activity of the final material administered, the dose rate equivalent in feed, the interval between the last dose and sacrifice, and an adequate description of sample storage conditions and intervals. If the storage interval exceeds 6 months, storage stability data may be required.

It is tentatively concluded that the nature of the residue in ruminants is adequately understood. The residue of concern is the parent, mepiquat choride. In addition to the parent, 4-hydroxymepiquat chloride and conjugates of 4-hydroxymepiquat chloride were found in liver (40% TRR) and in milk (about 20% TRR). However, these levels of metabolite are anticipated to be nondetectable at a 1X dietary exposure.

The analytical method, previously validated by an independent laboratory, should be submitted for Agency validation.

If you need additional information, please advise.

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Attachment: Mepiquat Chloride: Phase 5 - Reregistration Review, Residue Chemistry, Task 2B. 04/01/93.

cc: S. Funk, Mepiquat Chloride List B File, SF, RF, Circ., Acurex Corp.

RDI:E. Zager:09/28/92:
H7509C:CBRS:S.Funk:305-5430:CM#2:RM803-A:SF(0993.8/.9):09/27/93.

MEPIQUAT CHLORIDE
(Chemical Code 109101)
(CBRS Nos. 10685 and 11386;
DP Barcodes D183217 and D188232)

TASK 2B

Phase 5-
Reregistration Review
Residue Chemistry

April 1, 1993

Contract No. 68-DO-0142

Submitted to:

U.S. Environmental Protection Agency
Arlington, VA 22202

Submitted by:

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MEPIQUAT CHLORIDE

(Chemical Code 109101)

(CBRS Nos. 10685 and 11386; DP Barcodes D183217 and D188232)

PHASE 5 - REREGISTRATION REVIEW

RESIDUE CHEMISTRY

Task 2B

BACKGROUND

The Mepiquat Chloride Phase 4 Review (S. Funk, 1/15/91) specified residue chemistry data requirements including additional animal metabolism data, and residue analytical methods. Specifically, identification of the unknown (metabolite X) in goats' milk was required. Techniques such as NMR to locate the position of the hydroxyl group on the ring, or chemical dervivatization to eliminate one tautomer and provide a single isomer for characterization were suggested. As high levels of metabolite X were isolated from milk, the Phase 4 Review required data collection analytical methods for its determination in milk (after it is completely identified), and indicated that if it were determined to be a regulated metabolite, regulatory methods would also be required.

BASF Corporation (1991; MRIDs 42394301 through -04) has submitted supplements to animal metabolism studies (1988-89; MRIDs 41585201 through -06) screened in Phase 4, and an analytical method, including radiolabel validation. These data are reviewed here to determine their adequacy in fulfilling outstanding residue chemistry data requirements. These Conclusions and Recommendations are intended to update the status of metabolism and feeding studies on mepiquat chloride, as well as radiolabeled validation of residue analytical methods.

The qualitative nature of mepiquat chloride metabolism is adequately understood in plants, but not in animals. Cotton plant metabolism data (R. Perfetti, CBRS No. 10229; 3/5/93) indicate that the parent compound, mepiquat chloride, is the residue of concern, accounting for >90% of the total radioactive residue in cotton forage and cottonseed.

Method I in PAM, Vol. II, is described as very specialized and having recoveries in the 50% range, but is considered adequate for plant and animal tolerance enforcement purposes. Tolerances for residues of mepiquat chloride in plant and animal commodities are currently expressed in terms of N,N-dimethylpiperidinium chloride (40 CFR §180.384).

There are no established or proposed Codex MRLs for mepiquat chloride. Therefore, no compatibility questions exist with respect to U.S. tolerances and Codex.

CONCLUSIONS

1a. The ruminant metabolism studies are adequate pending submission of the in-life and storage information for the samples generated for the radiolabled validation (1991; MRID 42394303), and analyzed for metabolism purposes (1991; MRID 42394301). The required information includes: the specific activity of the final material administered, the dose rate equivalent in feed, the interval between the last dose and sacrifice, and an adequate description of sample storage conditions and intervals. If the storage interval exceeds 6 months, storage stability data may be required.

1b. In the original ruminant metabolism study (1988-89; MRIDs 41585204 through -06) the major residue identified in goat tissues dosed at 300x was unchanged parent ranging from 79.3% in omental fat to 95.3% in kidney. Methyl piperidine was also identified in each tissue ranging from 0.4% of the TRR in liver to 9% in renal fat. The majority of radioactive residues in milk samples was associated with the lactose fraction (35.7%, 0.076 ppm). Unchanged parent was detected at 0.029 ppm (13.6% of the TRR). In addition, 19.7% of the TRR (0.042 ppm) was putatively characterized as metabolite X, an α-monohydroxylated mepiquat chloride galactose conjugate. A total of 85.4% of the TRR in milk was characterized, including metabolite X.

1c. The current ruminant metabolism submission (1991; MRID 42394301) identified a different liver metabolite profile (a decrease in percent of unchanged parent, 80% of TRR, 10.4 ppm) from that seen in the initial study in liver samples. 4-Hydroxymepiquat glycerophosphate (metabolite Y) accounted for 34.5% of the TRR (5.985 ppm), unconjugated 4-hydroxymepiquat chloride accounted for 5.3% of the TRR (0.917 ppm), and unchanged parent accounted for 54.1% of the TRR (9.383) in these liver samples. In consideration of the exaggerated treatment rate (300X), of the absence of 4-hydroxymepiquat and/or its conjugates in other tissues and in poultry commodities, and the very low level in milk (<0.09 ppm, see 1d), the hydroxy metabolite will not be included in the residue of concern.

1d. The registrant has identified metabolite X in milk as 4-hydroxymepiquat galactose, and comprising 7.8-24.0% TRR (0.007-0.081 ppm) of the milk samples analyzed. In consideration of the exaggerated treatment rate (300X) and the low concentrations of 4-hydroxymepiquate galactose found (<0.09 ppm), the hydroxy metabolite will not be included in the residue of concern.

1e. Pending submission of the data/information requested in 1a., it is tentatively concluded that the residue of concern in ruminant commodities is the parent, mepiquat chloride. The compound 4-hydroxymepiquat chloride and its conjugates are anticipated to be present at levels below limits of detection for all commodities, assuming a limit of detection of 0.05 ppm.

2a. Poultry metabolism data (1988-89; MRIDs 41585201 through -03) are adequate. The major residue identified in eggs and tissues from hens treated at >800x is unchanged parent (85.9-98.5% of the TRRs).

2b. It is concluded that the residue of concern in poultry commodities is the parent, mepiquat chloride.

3a. The submitted radiolabeled validation data (1991; MRIDs 42394302 through -04) were generated in a separate goat ^{14}C-dosing study because there was insufficient material remaining from the original metabolism studies. These data validating a BASF ion chromatography method are adequate pending submission of all of the in-life information from the new study. The recoveries indicate that BASF method 286 adequately recovers residues of the parent mepiquat chloride from animal tissues.

3b. Recovery data for method 286 for fortified animal commodities are adequate for mepiquat chlorie (0.05, 1.0, 5.0 ppm) in poultry muscle, liver, skin, and fat and in ruminant milk, muscle, fat, liver, and kidney. Recovery was marginal for eggs (59% - 99%).

3c. The registrant must perform an independent laboratory validation for BASF method 286. The final report must include a complete copy of the method for purposes of Agency validation.

4. The Phase 4 review specified that feeding studies are required with cattle and poultry dosed for at least 28 days at levels based on the latest crop residue data available or to be generated.

Recommendation

CBRS recommends that the registrant be requested to supply the additional information for the ruminant metabolism study indicated in Conclusion No. 1a. The ruminant study is not acceptable, but may be upgraded. The poultry metabolism study is acceptable. Pending receipt of the additional information, it is tentatively concluded that the nature of the resiue in poultry and ruminants is adequately understood for purposes of the reregistration of mepiquat chloride and that the residue of concern consists of parent only, mepiquat chloride.

DETAILED CONSIDERATIONS

Qualitative Nature of the Residue in Animals

Ruminants. (1988-89 Studies)

The following discussion addresses a goat metabolism study (1988-89; MRIDs 41585204 through -06) and subsequent metabolite characterization of milk residues (1991; MRID 42394301) submitted in response to the Phase 4 screening of the original study. In addition, metabolite analyses were conducted on liver samples generated for the radiolabel method validation study.

BASF Corporation submitted data (1988-89; MRIDs 41585204 through -06) pertaining to the metabolism of [^{14}C]mepiquat chloride in lactating goats. One lactating goat (goat-1) received 954 mg of α, α'-labeled [^{14}C]mepiquat chloride (specific activity of dosed material 0.44 mCi/mmol; radiochemical purity of 99%) equivalent to approximately 798 ppm in feed (approximately 300x; see Table 1 for calculations) for 5 days. Milk samples were collected twice daily. Animals were sacrificed approximately 6 hours after the last dose. Samples were stored frozen $\leq$-18 °C for approximately 8 months prior to initiation of analyses.

The registrant stated that, because of the low specific activity of the dosing material in the first dosing, large amounts of matrix extracted with radiolabeled residues hindered characterization of residues. Therefore, a second goat (goat-2) was dosed similarly to goat-1 except with [^{14}C]mepiquat chloride at a higher specific activity (1000 mg; specific activity 1.49 mCi/mmol; radiochemical purity of 97.4%). Samples were stored frozen $\leq$-18 °C for approximately one month prior to initiation of analyses.

Table 1. Calculation of maximum theoretical dietary exposure in cattle[a].

Feed	Tolerance	Percent in Feed	Exposure (ppm)
Cottonseed	2	25	0.5
Cotton hulls	2[b]	15	0.3
Cotton forage	3	40	1.2
Cottonseed, meal	3	15	0.5
Other	0	5	0
Total	--	100	2.5 = 1x

[a]Not a realistic diet.
[b]RAC tolerance; no feed additive tolerance established.

Total Radioactive Residues (TRR)

The TRRs in milk samples were determined by direct LSS, and TRRs in tissues were determined by combustion followed by LSS. A summary of the TRRs and reported detection limits is provided in Table 2. The TRRs in milk samples collected from goat-1 increased from 0.264 ppm on day-1 to 0.585 ppm on day-5 and did not plateau. The TRRs in milk samples collected from goat-2 increased from 0.084 ppm on day-1 to 0.272 ppm on day-5 and did not plateau.

Extraction/Hydrolysis/Characterization of Residues

Residues were extracted from milk (milk-1) and tissues from goat-1 into MeOH. The MeOH extracts were characterized first by purification using cation exchange (SCX) chromatography, then alumina chromatography (except milk) followed by TLC analysis using two different solvent systems. The distribution of TRR in goat milk and tissues are presented in Table 3. Characterization results presented in Table 4 are averages from the two TLC analyses.

Residues were also extracted from goat-1 tissues into water (kidney 101.5 % TRR, liver 94.6% TRR, loin muscle 93.3% TRR, round muscle 96.9% TRR, omental fat 75.2% TRR, renal fat 93.2% TRR). The aqueous extracts were only used for determination of extractability of radioactive residues and were not characterized further.

Because large portions of radioactive residues were not characterized in milk-1, another milk sample (milk-2; day-4; goat-2) was analyzed. This sample was treated with H_3PO_4 (pH 4.2) to precipitate proteins and lipids, leaving an aqueous fraction (A1). Lipids were removed from the solids by extracting with MeOH and MeOH/chloroform (1:3; v:v), leaving a solid protein fraction. An aliquot of the aqueous fraction A1 was hydrolyzed with β-galactosidase in potassium phosphate buffer (pH 7) overnight at 37 °C, then incubated with baker's yeast overnight at 27 °C. As a result, 0.083 ppm (39% TRR) was extractable into dipicrylamine (DPA)/dichloromethane (DCM).

Table 2. Summary of TRRs in milk and tissues from goats dosed at 400x with [^{14}C]mepiquat chloride

Substrate	Dosing	Collection day	TRR (PPM); (second analysis)[a]	Detection Limit (PPM)
Milk	1	1 2 3 4 5	0.264 0.341 0.387 0.391 (0.331) 0.585	0.012
	2	1 2 3 4 5	0.084 0.120 0.166 0.196 (0.213) 0.272	
Kidney	1	sacrifice	18.2 (19.518)	0.042
Liver	1	sacrifice	12.2 (12.953)	0.044
Loin muscle	1	sacrifice	1.77 (2.010)	0.042
Round muscle	1	sacrifice	2.83 (3.055)	0.044
Omental fat	1	sacrifice	0.135 (0.145)	0.018
Renal fat	1	sacrifice	0.187 (0.200)	0.018

[a]These values were used to calculate %TRRs in subsequent analyses.

The DPA/DCM fraction was analyzed by TLC revealing unchanged parent (16% ot the TRR, 0.034 ppm) and an aglycone released from a galactose conjugate (20% of the TRR, 0.043 ppm).

Another aliquot of the aqueous fraction (A1) was mixed with an excess of acetone to precipitate carbohydrates (P1); the remaining aqueous phase was partitioned with MeOH/chloroform (9:1; v:v) to remove remaining lipids. The fractions were then separated into chloroform, containing lipids, and a MeOH fraction that was partitioned with DPA. The DPA-residues were then analyzed by TLC. TLC analysis identified unchanged parent (0.029 ppm, 13.6% TRR).

An aliquot of the precipitated carbohydrates (P1) was redissolved in water (A2), residual proteins were removed by centrifugation, and residues were partitioned from one aliquot of A2 into DPA/DCM (no measurable radioactivity found). Another aliquot of A2 was reacted with dinitrophenylhydrazine hydrochloride/sodium acetate solution at 32 °C for 3 hours. The resulting lactose osazone was recrystallized until constant specific radioactivity was achieved (characterized as lactose 0.076 ppm, 35.7 % TRR). A third aliquot of A2 was first hydrolyzed with β-galactosidase in potassium phosphate buffer (pH 7) overnight at 37 °C,

then incubated with baker's yeast overnight at 27 °C. The carbon dioxide formed was trapped and then determined by LSS. Yeast cells were removed, residues were partitioned into DPA/DCM, and the cleaved galactose conjugate (metabolite X) was analyzed by TLC. The aglycone was identified as monohydroxylated mepiquat chloride (0.042 ppm, 19.7% TRR).

For further characterization of metabolite X (not shown in Table 3), the registrant redissolved another aliquot of the precipitated carbohydrates (P1) in water, removed residual proteins by centrifugation, hydrolyzed the carbohydrate residues with 6M HCl for 3 hours, adjusted the pH to 7.2, partitioned the residues into DPA/DCM, cleaned up the residues using SCX chromatography, and isolated the residues using preparative HPLC. The isolated metabolite was subjected to LC-MS. The spectrum showed an intense molecular ion corresponding to a monohydroxylated mepiquat chloride. The registrant did not conclusively characterize the hydroxylated metabolite. However, they did eliminate two structures based on hypothesized instability and proposed another. Their hypotheses were: (i) that hydroxylation at either of the methyl groups would result in an unstable formaldehyde hemiaminal which would decompose to methylpiperidine; and (ii) that hydroxylation at the α-position on the ring is the most probable.

Table 3. Distribution and characterization/identification of TRR in goat milk and tissues.

Substrate	Fraction	%TRR	PPM	Characterization/Identification
Milk-1 (0 331)	MeOH	85.2	0.282	
	SCX column (HCL eluate)	50.5	0.167	0.146 ppm (44.1% TRR) identified as unchanged parent by TLC, and two resolved unknowns comprising a total of 0.021 ppm (6% TRR) determined by TLC
	Unextracted	14.8	0.049	none
Kidney (19.518)	MeOH	99.5	19.423	
	SCX column (HCL eluate)	97.2	18.975	
	Alumina column (acetone/MeOH eluate)	96.7	18.873	an average of 18.6 ppm (95.3% TRR) was identified as unchanged parent, and 0.255 ppm (1.3% TRR) identified as methyl piperidine by TLC using two different solvent systems
	Unextracted	0.5	0.098	none
Liver (12.953)	MeOH	94.3	12.219	
	SCX column (HCL eluate)	85.9	11.125	
	Alumina column (acetone/MeOH eluate)	80.6	10.435	an average of 10.377 ppm (80.1% TRR) was identified as unchanged parent, and 0.052 ppm (0.4% TRR) identified as methyl piperidine by TLC using two different solvent systems
	Unextracted	5.7	0.738	none
Loin muscle (2.010)	MeOH	95.7	1.924	
	SCX column (HCL eluate)	92.1	1.851	
	Alumina column (acetone/MeOH eluate)	93.5	1.880	an average of 1.866 ppm (92.8% TRR) was identified as unchanged parent, and 0.012 ppm (0.6% TRR) identified as methyl piperidine by TLC using two different solvent systems
	Unextracted	4.3	0.086	none
Round muscle (3.055)	MeOH	98.0	2.994	
	SCX column (HCL eluate)	92.8	2.834	
	Alumina column (acetone/MeOH eluate)	88.2	2.694	an average of 2.582 ppm (84.5% TRR) was identified as unchanged parent, 0.061 ppm (2.0% TRR) identified as methyl piperidine, and 0.047 ppm (1.5% TRR) was characterized as other unknowns by TLC using two different solvent systems
	Unextracted	2.0	0.061	none

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(continued)

Table 3. (continued)

Substrate	Fraction	%TRR	PPM	Characterization/Identification
Omental fat (0.145)	MeOH	87 6	0.127	
	SCX column (HCL eluate)	89.0	0.129	
	Alumina column (acetone/MeOH eluate)	84 1	0.122	an average of 0.115 ppm 179.3% TRR) was identified as unchanged parent, 0.003 ppm (2.1% TRR) identified as methyl piperidine, and 0.005 ppm (3.4% TRR) was characterized as other unknowns by TLC using two different solvent systems
	Unextracted	12.4	0.018	none
Renal fat (0.200)	MeOH	89.0	0.178	
	SCX column (HCL eluate)	91.0	0.182	
	Alumina column (acetone/MeOH eluate)	88.0	0.176	an average of 0.159 ppm (79.5% TRR) was identified as unchanged parent, and 0.018 ppm 19% TRR) identified as methyl piperidine by TLC using two different solvent systems
	Unextracted	11.0	0.022	none
Milk-2 (0.213)	Protein/lipid phosphoric acid precipitate	13.6	0.029	(i) proteins/lipids precipitated by adjusting pH to 4.2; (ii) lipids (7% TRR; 0.015 ppm) partitioned into MeOH/$CHCl_3$ from protein precipitate (identification not confirmed); (iii) remaining solids (6.6% TRR, 0.014 ppm) characterized as proteins (identification not confirmed)
	Aqueous	84.0	0.179	(i) an aliquot of the aqueous fraction (A1) was incubated with galactosidase and yeast, 0.083 ppm (39% TRR) was extractable into DPA/DCM; TLC analysis estimated 0.034 ppm (16% TRR) as unchanged parent, and 0.043 ppm (20% TRR) as unknown aglycone, while 1.4% of the TRR remained at the origin (ii) another aliquot of the aqueous phase was treated with acetone to precipitate carbohydrates including lactose
	Acetone filtrate	24.4	0.052	(i) partitioned with chloroform/MeOH; (ii) chloroform (0.9% TRR; 0.002 ppm), characterized as lipid fraction (no confirmation provided); (iii) MeOH fraction (24.4% TRR; 0.052 ppm) further partitioned with DPA; TLC analysis of resulting fraction identified 0.029 ppm (13.6% TRR) as unchanged parent.
	Carbohydrate precipitate (aqueous solution)	59.1	0.126	the carbohydrate precipitate (P1) was redissolved in water to give the fraction A2; 0.004 ppm (1.9% TRR) remained as solid protein
	dinitrophenyl-hydrazine reactant	35.7	0.076	lactose fraction; osazone recrystallized to contain specific activity
	galactosidase/yeast hydrolysate	20.2	0.043	0.042 ppm (19.7% TRR) partitioned into DCP/DCM; hydrolysis product identified as galactose conjugate designated metabolite X, a monohydroxylated mepiquat chloride by LC-MS

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Table 4. Characterization of residues in ruminant milk samples[a].

Metabolite/Fraction	Milk-1		Milk-2	
	%TRR	PPM	%TRR	PPM
Mepiquat chloride	44.1	0.146	13.6-16.0	0.029-0.034
Metabolite X	--	--	19.7	0.042
Lactose	--	--	35.7	0.076
Proteins	--	--	8.5	0.018
Lipids	--	--	7.9	0.017
Unknowns	6.0	0.021	1.4	0.003
Total Characterized	44.1	0.146	85.4	0.182

[a]Results are the average of two TLC analyses.

Greater than 80% of the TRRs in kidney, liver, muscle, and fat were adequately identified. The major residue identified was unchanged parent in each kidney (95.3%), liver (80.1%), loin muscle (92.8%), round muscle (84.5%), omental fat (79.3%), and renal fat (79.5%). Methyl piperidine was also identified in kidney (1.3%), liver (0.4%), loin muscle (0.6%), round muscle (2.0%), omental fat (2.1%), and renal fat (9.0%). In addition, two unknowns were found in round muscle (1.5% TRR) and omental fat (3.4% TRR).

As summarized in Table 4, the majority of radioactive residues in milk (milk-2) were associated with the lactose fraction (35.7%). Unchanged parent accounted for 13.6% of the TRR. In addition, 19.7% of the TRR was putatively characterized as a α-monohydroxylated mepiquat chloride galactose conjugate, designated (metabolite X). Fractions characterized as lipid and protein accounted for 7.9 and 8.5% of the TRR, respectively. A total of 85.4% of the TRR in milk was characterized, including metabolite X.

The Mepiquat Chloride Phase 4 Review (S. Funk, 1/15/91) specifically required identification of the ring hydroxylated mepiquat chloride metabolite in goats' milk. Techniques such as NMR to locate the position of the hydroxyl group on the ring, or chemical derivatization to eliminate one tautomer providing a single isomer for characterization were suggested. As significant levels of a hydroxylated mepiquat chloride metabolite were putatively identified in milk, the Phase 4 Review required data collection analytical methods for its determination in milk, and noted that a regulatory method might be required.

Ruminants. (1991 Studies)

In response to Phase 4 data requirements, BASF Corporation submitted additional data (1991; MRID 42394301) pertaining to the metabolism of [^{14}C]mepiquat chloride in lactating goats. The submitted data include (i) further characterization of metabolite X in milk as requested by the Phase 4 review and (ii) investigation of a different liver metabolite profile discovered during a radiolabel method validation study.

During a radiolabeled validation (1991; MRID 42394303) of BASF method 286 the registrant discovered that new liver samples (generated specifically for radiolabeled validation study) contained residues of parent mepiquat chloride that only accounted for 47% of the TRR where as higher levels (80% of the TRR) were found in the original study. The registrant concluded that a different metabolite composition was present in the new liver sample, and that further investigation of the new liver sample was justified. These data are summarized below along with further characterization of metabolite X in milk.

Three different composite milk samples were analyzed. Two were composites of samples from the original metabolism study (1988-89; MRIDs 41585204 through -06). The third sample was composed of day-5, -6 and -7 milk samples generated for the radiolabeled validation of BASF method no. 286 (1991; MRID 42394303).

The liver samples and the third milk sample, generated for the radiolabel method validation, were subjected to further analysis and reported in the current submission. The goats reportedly received nominal daily doses of 1000 ppm α, α'-labeled [^{14}C]mepiquat chloride (specific activity 2.45 x 10^7 dpm/mg; radiochemical purity of 97.4%) for 8 days. The registrant did not clearly indicate the specific activity of the final material dosed, the dose rate equivalent in feed, or the interval between the last dose and sacrifice; this information is required.

Samples generated for the original metabolism study (1988-89; MRIDs 41585204 through -06) were stored for up to 27 months. The available information indicates that samples generated for the radiolabel validation were stored for at least 12 months; however, the actual storage intervals and conditions were not provided for these samples. These data are required.

Total Radioactive Residues (TRR)

The TRR levels in each of three composite milk samples (1; 0.469 ppm, 2; 0.091 ppm, 3; 0.338 ppm), and the liver sample (17.332 ppm) were determined either by direct LSS or by combustion and subsequent LSS.

Extraction/Hydrolysis of Residues

Liver.

Three different aliquots of liver were extracted using three different procedures (Liver-1 through -3, Table 5) in an attempt to optimize extraction and clean-up. A description encompassing all three procedures is provided in the following paragraphs.

Residues in liver (Liver-1 through -3) were extracted into MeOH, concentrated, redissolved in water/acetone/methanol (1/1/3; v/v/v), and applied to a cation exchange column (SCX column). Residues were eluted sequentially in acetone, water, and aqueous HCl, and the aqueous (A1) and HCl fractions were analyzed by TLC (only Liver-1) using two different solvent systems.

One aliquot (Liver-2) of the aqueous fraction (A1) was concentrated, redissolved in MeOH, hydrolyzed with 0.5M HCl at 100 °C for 3 hours, neutralized, concentrated and redissolved in water/acetone/methanol (1/1/3; v/v/v), and applied to a SCX column. Residues were again eluted sequentially in acetone, water, and aqueous HCl. An aliquot of the resulting aqueous acid fraction, which contained the highest level of radioactivity, was analyzed by TLC. Another aliquot was also concentrated, redissolved in 0.25M sodium phosphate buffer (pH 7.3), residues were partitioned into DCM containing DPA, then into aqueous HCl and analyzed by MS.

A second aliquot (Liver-2) of the aqueous fraction A1 was concentrated, redissolved in acetone/water (20/1; v/v), and applied to a phenyl cleanup cartridge. The residues were eluted (E1) with aqueous solvents. An aliquot of the aqueous eluate (E1) was concentrated, redissolved in acetone, and the resulting precipitate was acetylated and subsequently chromatographed using HPLC. The resulting fraction that contained the highest level of radioactivity was analyzed by MS, acid hydrolyzed, and analyzed by HPLC and MS.

An aliquot (Liver-3) of the aqueous fraction (A1) was concentrated, and residues were redissolved in water, applied to a phenyl cleanup cartridge, and eluted in water (E4). One aliquot of the water fraction (E4) was acid hydrolyzed, concentrated and redissolved in water/acetone/methanol (1/1/3; v/v/v), and applied to a SCX column. Residues were eluted sequentially in acetone, water, and aqueous HCl. The aqueous acid fraction, which contained the highest level of radioactivity, was applied to a phenyl cleanup cartridge, and residues were eluted in water. The resulting residues were hydrolyzed and applied first to a SCX column then to a phenyl cleanup cartridge. The fraction containing the highest level of radioactivity was acetylated, again applied to a SCX column, and the two fractions containing the highest level of radioactivity were analyzed by MS. A second aliquot of fraction E4 was chromatographed using HPLC, and the fraction containing the highest level of radioactivity was rechromatographed using a different solvent system. The resulting fraction containing the highest level of radioactivity was analyzed by MS, acetylated and further fractionated

using HPLC. The resulting two major fractions containing the most radioactivity were analyzed by MS.

Milk.

Metabolite X was isolated from each of three milk samples by a procedure that was similar to the extraction procedure that isolated the metabolite X aglycone in the original metabolism study (1989; MRID 41585204). Specifically, milk proteins and lipids were precipitated with phosphoric acid and then separated by centrifugation. The supernatant was mixed with acetone to precipitate carbohydrates which were separated and then washed with acetone. The precipitated carbohydrates were hydrolyzed with 0.5M HCl for 3 hours at 100 °C. Metabolite X remaining in the hydrolysate was partitioned into DPA/DCM, and subsequently partitioned into aqueous HCl. The hydrolysate (aglycone) of metabolite X was co-chromatographed with hydrolyzed metabolite Y. The results indicated that the hydrolysis products were identical.

Table 5. Distribution and characterization/identification of TRR in goat liver.

Substrate	Fraction	%TRR	PPM	Characterization/Identification
Liver-1 (17.332)	MeOH	96.2	16.671	
	SCX column (acetone and application eluates)	3.1	0.511	none
	SCX column (aqueous eluate)	21.0	3.641	3.641 ppm (21.0% TRR) was designated metabolite Y (a conjugate) by TLC using two different solvent systems
	SCX column (HCL eluate)	73.0	12.645	9.383 ppm (54.1% TRR) identified as unchanged parent by radio-TLC, 2.344 ppm (13.5% TRR) designated metabolite Y, and 0.917 ppm (5.3% TRR) characterized as unconjugated metabolite Y by TLC using two different solvent systems
Liver-2 (17.332)	MeOH	95.9	16.623	
	SCX column (acetone and application eluates)	4.6	0.792	none
	SCX column (aqueous eluate)	22.3	3.873	Fraction A1
	Acid hydrolysate	22.4	3.890	
	SCX column (HCl eluate)	20.9	3.615	Fraction analyzed by TLC; results not reported
	DPA/HCl	18.0	3.118	Residues in the resulting organic phase were subsequently partitioned into aqueous HCl and analyzed by MS. MS results showed intensive ion (m/z 130) in agreement with hydroxylated mepiquat chloride ion
	Phenyl cartridge (aqueous eluate)	21.6	3.75	Fraction E1
	Acetone precipitate	19.4	3.361	
	Acetate product	19.9	3.454	An aliquot of the resulting acetate residues was subjected to HPLC analysis to further separate the residues, and the main fraction was analyzed by MS. Another aliquot of the acetate residues was hydrolyzed and the product was analyzed by HPLC and MS.
	SCX column (HCL eluate)	58.9	10.213	none
Liver-3 (17.332)	MeOH	96.9	16.788	
(continued)	SCX column (acetone and application eluates)	6.5	1.13	none

(continued).

Table 4. (continued)

Substrate	Fraction	%TRR	PPM	Characterization/Identification
Liver-3 (cont.) (17.332)	SCX column (aqueous eluate)	23.0	3.993	Fraction A1
	Phenyl cartridge (aqueous eluate)	22.3	3.874	Fraction E4
	Acid hydrolysate	ND*	ND	An aliquot was HPLC chromatographed, the main fraction was again HPLC chromatographed (different system) and one aliquot of the main was analyzed by MS (results agreed with assumption of a glycerophosphoric acid conjugate of 4-hydroxymepiquat chloride), another aliquot was acetylated, HPLC chromatographed, and the two major fractions were analyzed by MS which confirmed above assumption. The main fraction showed two molecular ions indicating a diacetate.
	SCX column (HCl eluate)	21.8	3.787	
	Phenyl cartridge (aqueous eluate)	20.0	3.478	(i) cochromatography with 4- and 3-hydroxymepiquat chloride reference standards indicated that Metabolite Y is a 4-hydroxymepiquat chloride conjugate; (ii) the eluate was chromatographed by HPLC, the main fraction was acetylated and chromatographed on SCX column, the two main fractions were analyzed by MS. Comparison of the mass spectrum of a 4-acetoxy-mepiquat chloride standard with that of the hydrolyzed/acetylated metabolite Y was "similar"
	SCX column (HCL eluate)	54.7	9.476	none

*ND=not determined.

Characterization of Residues

Mass Spectral analyses of liver sample extracts identified, conjugated 4-hydroxymepiquat glycerophosphate (metabolite Y), which accounted for 34.5% of the TRR (5.985 ppm), and unconjugated 4-hydroxymepiquat chloride, which accounted for 5.3% of the TRR (0.917 ppm). Hydroxylation at the 4-position was confirmed by chromatographic comparison with synthetic 3- and 4-hydroxymepiquat chloride standards. 2-Hydroxymepiquat chloride was excluded because of instability of the isomer. The parent mepiquat chloride accounted for 54.1% of the TRR (9.383 ppm) in liver. The total percent of radioactive residues identified in goat liver was 93.9% (16.285 ppm). Chromatographic comparison of hydrolyzed metabolite X from milk with the hydrolyzed liver extracts indicated that metabolite X is also a 4-hydroxymepiquat chloride conjugate and comprised 7.8-24.0% TRR (0.007-0.081 ppm) of the milk samples analyzed. Metabolite X in milk was shown in the original study to be a galactose conjugate in contrast to liver, in which the 4-hydroxy metabolite is conjugated with glycerophosphoric acid.

The registrant proposed that mepiquat chloride undergoes oxidation to the 4-hydroxymepiquat chloride and subsequent conjugation with galactose or glycerophosphoric acid. Molecular structures and chemical names of mepiquat chloride and known metabolites are summarized in Table 6.

The submitted ruminant metabolism data are adequate pending submission of additional information from the radiolabel validation study, which yielded the samples for metabolite analyses. Specifically, the registrant must submit the specific activity of the final material dosed, the dose rate equivalent in feed, the interval between the last dose and sacrifice, and the actual storage intervals and conditions.

Table 6. Molecular structures and chemical names of mepiquat chloride and known metabolites.

Chemical Name (Common Name)	Structure	Matrices/MRID
N,N-dimethylpiperidinium chloride (mepiquat chloride)	H_3C CH_3 N Cl^-	cotton forage 42330804 cottonseed 42330804 goat: 41585206 kidney, liver, muscle, fat, and milk goat: 42394301 liver and milk hen: eggs and tissues 41585201
4-hydroxy-1,' dimethylpiperidinium chloride (4-hydroxymepiquat chloride)	OH N H_3C CH_3 Cl^-	goat: 42394301 liver
1-Methylpiperidine (methylpiperidine)	N CH_3	goat: 41585206 kidney, liver, muscle, and fat hen: (putative) muscle and skin 41585201
4-hydroxymepiquat glycerophosphate conjugate (metabolite Y)		goat: 42394301 liver
4-hydroxymepiquat galactose conjugate (metabolite X)		goat: 42394301 milk

In addition to the above, the following reference standards were used: 1-methylpiperidine, piperidine, piperidine, and 3-hydroxy-1,1-dimethylpiperidinium chloride.

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Poultry. BASF Corporation submitted data (1988-89; MRIDs 41585201 through -03) pertaining to the metabolism of [^{14}C]mepiquat chloride in laying hens. Twenty seven hens (hen group-1) received 36.1 mg of α, α'-labeled [^{14}C]mepiquat chloride (specific activity 0.48 mCi/mmol; radiochemical purity of 99%) equivalent to approximately 254 ppm in feed (847x; see Table 7 for maximum theoretical dietary exposure calculations) for 6 days. Eggs were collected twice daily and then pooled for the day. Animals were sacrificed approximately 6 hours after the last dose. Samples were stored frozen ≤-18 °C for approximately 9 months prior to initiation of analyses.

The registrant stated that, because of the low specific activity of the dosing material in the first dosing group, large amounts of matrix material were coextracted with radiolabeled residues hindering characterization of residues. Therefore, a second group of hens (hen group-2) was dosed similarly to group-1 except with [^{14}C]mepiquat chloride at a higher specific activity (35 mg; specific activity 1.85 mCi/mmol; radiochemical purity of 97%). Samples were stored frozen ≤-18 °C for up to 6 months prior to initiation of analyses.

Table 7. Calculation of maximum theoretical dietary exposure in poultry.

Feed	Tolerance	Percent in Feed	Exposure (ppm)
Cottonseed meal	2[a]	10	0.2
Cottonseed soapstock	2[a]	5	0.1
Other	0	85	0
Total	--	100	0.3 = 1x

[a]RAC tolerance; no feed additive tolerance established.

Total Radioactive Residues (TRR)

The TRR levels from group-1 and -2 hens and reported detection limits are summarized in Table 8. TRRs were determined by direct liquid scintillation spectrometry (LSS) or by combustion and subsequent LSS. The registrant performed more than one TRR analysis on each substrate. The results presented here are from the registrants tables A2.3, A2.4, and A2.5; values reported in the registrants Tables 2 and 3 may differ may from the presented results. The presented results were used to determine %TRRs in subsequent Tables.

Table 8. Total radioactive residues (TRR) in poultry tissues and eggs from hens dosed with mepiquat chloride at 254 ppm.

Matrix	TRR (ppm)			
	Hen Group-1; 0.48 mCi/mmol	Detection Limit (Hen Group-1)	Hen group-2; 1.85 mCi/mmol	Detection Limit (Hen Group-2)
Kidney	2.803	0.046	4.551	0.008
Liver	1.356	0.046	0.996-1.449	0.008
Muscle			0.247[a]	0.010
Thigh	0.324	0.088		
Breast	0.297	0.052		
Fat	0.227	0.076	4.14[b]	0.022
Skin	0.525	0.040	1.121[c]	0.014
Eggs (day):		0.042		
0-2	<0.008			
3	0.305			
4	0.583			
5	0.908		1.018	
6	1.272			

[a]Type unspecified. [b]Fat solids only. [c]Skin solids only; tissue fluid 2.076 ppm.

Extraction/Characterization of Residues

Eggs.

Residues were extracted from eggs (hen groups 1 and 2) into MeOH. Aliquots of the MeOH fractions from group 1 were was adjusted to 7.4 pH (E1). Residues from hen group-2 eggs were applied to a cation exchange column (SCX), and the resulting acetone/HCl eluate was adjusted to pH 7.4 (E2). E1 and E2 fractions were applied to an extrelute column and eluted in a DPA/DCM solution. Residues were then partitioned into aqueous HCl and analyzed by TLC using two solvent systems. These results are summarized in Table 9 below.

Tissues.

Residues were extracted from tissues (liver, kidney, muscle, fat, and skin) of group-1 hens into MeOH. Aliquots of the MeOH fractions were adjusted to pH 7.4 (T1). T1 fractions from liver, thigh, and breast muscle samples were applied to an extrelute column, eluted in a DPA/DCM solution, partitioned into aqueous HCl, and further purified by column chromatography (Sephadex LH 20); residues were eluted in MeOH (F1). T1 fractions from fat and skin were purified by column chromatography (silica gel); residues were eluted sequentially in acetone, acetone/MeOH (7/3; v/v), acetone/MeOH (1/1; v/v), MeOH, and methanolic HCl (F2). The T1 kidney fraction was applied to a cation exchange column

(Dowex), and residues were eluted sequentially in acetone and 1M HCl (F3). Residues in the final fractions (F1 through 3) were then analyzed by TLC using two solvent systems.

Residues were extracted from hen group-2 samples of liver, kidney, muscle, and homogenized skin and fat into saturated aqueous NaCl. Residues were then partitioned into acetone, and the precipitates were removed by filtration, resulting in acetone (A1) fractions and precipitated residues. In addition, the liver residues were then applied to a cation exchange column (SCX), and eluted in acetone (A2). A1 and A2 fractions were analyzed by TLC using two different solvent systems. Fat and skin samples from hen group-2 were homogenized and a liquid lipid layer was separated. Fluid from the skin was applied to SCX column and residues were eluted with aqueous HCl; TLC analysis (silica gel and alumina) identified 1.975 ppm (95.2% TRR) as unchanged parent. The fat lipid fraction was not further characterized (0.022 ppm).

Table 9. Distribution and characterization/identification of TRR in eggs and hen tissues.

Substrate	Fraction	%TRR	PPM	Characterization/Identification
Eggs (Hen Group-1) (0.908)	MeOH	91.5	0.831	adjusted to pH 7.4: fraction E1
	Extrelute column (DPA/DCM eluate); HCl/H_2O partition	71.6	0.650	TLC analysis (silica gel) identified 0.650 ppm (71.6% TRR) as unchanged parent; TLC analysis (alumina) identified 0.623 ppm (68.6% TRR) as unchanged parent and 0.026 ppm (1.5% TRR) as unknowns
	Unextracted	4.4	0.040	none
Eggs (Hen Group-2) (1.0181) (day-5)	MeOH	92.0	0.937	
	Cation exchange (SCX) (HCl eluate)	84.4	0.861	adjusted to pH 7.4: fraction E2
	Extrelute column (DPA/DCM eluate); HCl/H_2O partition	85.9	0.874	TLC analysis (silica gel) identified 0.874 ppm (85.9% TRR) as unchanged parent; TLC analysis (alumina) identified 0.874 ppm (85.9% TRR) as unchanged parent
	Unextracted	4.1	0.042	none
Liver (Hen Group-1) (1.3561	MeOH	89.0	1.206	adjusted to pH 7.4: fraction T1
	Extrelute column (DPA/DCM eluate);H_2O/HC(	55.9	0.758	
	LH20 column (methanol eluate)	62.0	0.841	Fraction F1: TLC analysis (silica gel) identified 0.813 ppm (59.9% TRR) as unchanged parent, and 0.028 ppm (2.1% TRR) as an unknown; TLC analysis (alumina) identified 0.841 ppm (62.0% TRR) as unchanged parent
	Unextracted	9.5	0.129	none
Liver (Hen Group-2) (1.083)	Saturated NaCl/H_2O	101.1	1.095	
	Acetone	87.9	0.951	Fraction A1
	SCX column (HCl eluate)	86.5	0.935	
	Acetone	89.9	0.972	Fraction A2: TLC analysis (silica gel and alumina) identified 0.964 ppm (89.1% TRR) as unchanged parent
	Unextracted/precipitated total	11.7	0.127	none
Kidney (Hen Group-1) (2.803)	MeOH	92.3	2.588	adjusted to pH 7.4: fraction T1
	SCX column (HCl eluate)	84.6	2.37(	TLC analysis (silica gel) identified 2.37 ppm (84.6% TRR) as unchanged parent
	Unextracted	8.9	0.248	none

(continued).

Table 9. (continued)

Substrate	Fraction	%TRR	PPM	Characterization/Identification
Kidney (Hen Group-2) (4.551)	Saturated NaCl/H_2O	93.1	4.238	
	Acetone	90.0	4.093	Fraction A1: TLC analysis (silica gel and alumina) identified 4.093 ppm (90.0% TRR) as unchanged parent
	Unextracted/precipitated total	9.4	0.428	none
Breast muscle (Hen Group-1) (0.297)	MeOH	91.0	0.270	adjusted to pH 7.4: fraction T1
	Extrelute column (DPA/DCM eluate);H_2O/HCl	78.9	0.234	
	LH20 column (MeOH eluate)	73.5	0.218	Fraction F1: TLC analysis (silica gel) identified 0.200 ppm (67.5% TRR) as unchanged parent, and 0.031 ppm (10.6% TRR) as methylpiperidine; TLC analysis (alumina) identified 0.187 ppm (63.2% TRR) as unchanged parent, 0.011 ppm (3.6% TRR) as methylpiperidine, and 0.030 ppm (11.2% TRR) as an unknown
	Unextracted	8.8	0.026	none
Thigh muscle (Hen Group-1) (0.324)	MeOH	90.7	0.294	adjusted to pH 7.4: fraction T1
	Extrelute column (DPA/DCM eluate);H_2O/HCl	68.9	0.223	
	LH20 column (MeOH eluate)	69.1	0.224	Fraction F1: TLC analysis (silica gel) identified 0.188 ppm (58.1% TRR) as unchanged parent, and 0.032 ppm (9.8% TRR) as methylpiperidine; TLC analysis (alumina) identified 0.198 ppm (61.2% TRR) as unchanged parent, 0.009 ppm (2.7% TRR) as methylpiperidine, and 0.013 ppm (4.0% TRR) as an unknown
	Unextracted	9.7	0.031	none
Muscle (Hen Group-2) (0.247)	Saturated NaCl/H_2O	96.0	0.237	
	Acetone	98.5	0.242	Fraction A1: TLC analysis (silica gel and alumina) identified 0.242 ppm (98.5% TRR) as unchanged parent
	Unextracted/precipitated total	7.7	0.019	none
Fat (Hen Group-1) (0.277)	MeOH	57.5	0.187	adjusted to pH 7.4: fraction T1
	Silica gel column (MeOH HCl eluate)	74.8	0.207	TLC analysis (silica gel) identified 0.207 ppm (74.8% TRR) as unchanged parent; TLC analysis (alumina) identified 0.189 ppm (58.2% TRR) as unchanged parent, and 0.018 ppm (5.5% TRR) as an unknown
	Unextracted	25.2	0.070	none

(continued).

Table 9. (continued)

Substrate	Fraction	%TRR	PPM	Characterization/Identification
Fat (Hen Group-2) (4.14)	Saturated NaCl/H_2O	94.7	3.927	
	Acetone	92.1	3.812	Fraction A1: TLC analysis (silica gel and alumina) identified 3.81 ppm (92.1% TRR) as unchanged parent
	Unextracted/precipitated total	1.5	0.062	none
Skin (Hen Group-1) (0.525)	MeOH	48.0	0.252	adjusted to pH 7.4: fraction T1
	Silica gel column (MeOH HCl eluate)	32.1	0.169	TLC analysis (silica gel) identified 0.169 ppm (32.1% TRR) as unchanged parent; TLC analysis (alumina) identified 0.156 ppm (29.7% TRR) as unchanged parent, 0.007 ppm (1.3% TRR) as methylpiperidine, and 0.005 ppm (1.0% TRR) as an unknown
	Unextracted	16.6	0.087	none
Skin (Hen Group-2) (1.121)	Saturated NaCl/H_2O	87.3	0.978	Fluid from the skin was applied to SCX column and residues were eluted with aqueous HCl (1.975 ppm, 95.2% TRR); TLC analysis (silica gel and alumina) identified 1.975 ppm (95.2% TRR) as unchanged parent
	Acetone	86.6	0.969	TLC analysis (silica gel and alumina) identified 0.969 ppm (86.6% TRR) as unchanged parent
	Unextracted/precipitate total	3.8	0.043	none

Characterization of Residues

TLC analyses of the group-2 hen sample fractions identified 85.9-98.5% of the TRRs in egg, liver, kidney, muscle, fat, and skin as unchanged parent. TLC analyses of group-1 hen sample fractions gave slightly different profiles. The major residue in each sample was unchanged parent, which accounted for 29.7% of the TRR in skin to 84.6% of the TRR in kidney. In addition to residues of parent, methylpiperidine residues were identified in breast muscle (3.6-10.6% TRR; 0.031-0.011 ppm) and thigh muscle (2.7-9.8% TRR; 0.009-0.032 ppm), and skin (1.3% TRR; 0.007 ppm). Unknowns found in the group-1 samples that were not found in the group-2 samples ranged from 1% of the TRR (0.005 ppm) in skin to 11.2% of the TRR (0.03 ppm) in breast muscle. Differences in metabolite profiles may have been due to differences in storage intervals. Group-1 hen samples were stored for 9 months were as group-2 samples were stored for only 6 months. The registrant attributed profile differences to cleaner extracts in the group-2 samples due to the administering of radiolabeled material with a higher specific activity.

The available poultry metabolism data are adequate. The major residue in eggs and tissues is unchanged parent.

Residue Analytical Method Validation

BASF tested their residue method 286 (1991; MRIDs 42394303 and -04) using tissues and milk from goats that reportedly received nominal daily doses of 1000 ppm of α, α'-labeled [^{14}C]mepiquat chloride (specific activity 2.45 x 10^7 dpm/mg; radiochemical purity 97.4%) for 8 days. Poultry tissues and eggs were also tested, although details of the in-life study were not provided. The registrant did not clearly indicate the specific activity of the final material dosed, the dose rate equivalent in feed, or the interval between the last dose and sacrificed for ruminants or poultry. In addition, for poultry, the number of days dosed, and the radiochemical purity were not provided.

Ruminant liver samples generated for these validation studies were also subject of a metabolism study (1991; MRID 42394301) reviewed in this document. Determination of the adequacy of those metabolism data is pending submission of the in-life information. In addition, these in-life data are required to determine if the new samples generated for these studies are representative of the samples used to delineate the nature of the residue in animals.

BASF method 286 is notably different from the PAM Vol. II Method I enforcement method. BASF method 286 involves extraction of residues by macerating the sample in a water/2M Hcl/acetone (100/1/200; v/v/v) solution. The extract is filtered, concentrated, and washed with DCM. The Ph of the acidic aqueous phase is adjusted to alkaline. Residues are partitioned into DCM containing dipicrylamine, then into acidic aqueous solution, concentrated, and applied to an alumina column. Residues are then eluted with acetone/methanol (95/5; v/v), concentrated, and determined by ion chromatography using conductivity detection. LSS results for each extraction step are compared with IC analysis of the final extract (values in shaded rows) in Table 10. The reported detection limit is 0.05 ppm for mepiquat chloride. Recoveries from samples fortified with mepiquat chloride are summarized in Table 11 below.

In addition, goat liver samples were concurrently analyzed using a modification of method 286 that involved extraction of residues into MeOH instead of water/Hcl/acetone. The methanol extraction procedure improved the extraction efficiency to 83.5% TRR (13.41 ppm) from 64.9% TRR (10.6 ppm). However, when residues were subsequently partitioned into acidified H_2O/acetone, precipitates were formed and the soluble residue levels dropped to 62.3% (10.0 ppm). This modification to BASF method 286 does not provide significant improvement to the liver extraction efficiency.

The registrant has shown that BASF method 286 adequately recovers residues of mepiquat chloride from animal tissues. The metabolism and validation studies concur that 46.7%-54.1% of the TRR in ruminant liver (generated for the validation study and analyzed by both metabolism and validation studies) is unchanged parent. However, the goat metabolism data also indicate that radioactive residues other than parent are present in liver and milk at levels >10% of the TRR.

If the 4-hydroxymepiquat metabolites identified in the goat metabolism study are found to be of toxicological concern, additional methodologies and radiolabeled validation data will be required. After all residues of toxicological concern have been identified, and adequate radiolabeled validation data have been submitted for those residues, the registrant must submit data from an independent laboratory validation of BASF method 286 The registrant must then submit a copy of the method (including any required modifications) for Agency validation so its adequacy as an enforcement method can be determined. The registrant must also submit the in-life information on the samples generated for this study as required in the nature of the residue section.

Table 10. Recovery of radioactive residues (TRR) from tissues, eggs and milk by BASF method 286 extraction procedures[a].

Fraction	LSS Analysis											
	Muscle		Liver		Fat		Kidney		Milk/Eggs		Skin	
	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM
Ruminant: Solids	6.1	0.22	31.5	5.14	17.1	0.25	4.2	0.73	8.9	0.04	--	--
Acidified H_2O/acetone	98.6	3.52	64.9	10.6	99.0	1.42	95.8	16.69	104.0	0.42	--	--
DCM	<DL	<DL	1.4	0.23	2.9	0.04	0.5	0.09	<DL	<DL	--	--
Acidic phase	102.7	3.66	67.8	11.07	103.8	1.49	98.8	17.21	71.8	0.29	--	--
Aqueous alkaline phase	2.0	0.07	12.6	2.06	6.2	0.09	4.8	0.83	38.5	.16	--	--
DPA/DCM	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	--	--
DCM	<DL	<DL	<DL	<DL	<DL	<DL	0.2	0.03	<DL	<DL	--	--
Acid aqueous phase	97.2	3.47	54.9	8.96	87.8	1.26	92.9	16.18	48.6	0.20	--	--
Final dilution; Acetone/methanol eluent[b]	78.8	2.81	46.7	7.63	72.9	1.05	80.5	14.02	19.9	0.08	--	--
Ion chromatographic analysis[c]	**76.5**	**2.73**	**46.7**	**7.62**	**79.9**	**1.15**	**85.5**	**14.9**	**18.2**	**0.07**	--	--
Poultry: Solids	10.6	0.025	23.7	0.33	1.3	0.03	8.0	0.36	5.2	0.03	1.7	0.02
Acidified H_2O/acetone	106.2[d]	0.25	88.1[d]	1.23	101.5	2.31	80.1	3.59	91.2	0.48	98.2	1.07
DCM	<DL	<DL	<DL	<DL	0.7	0.02	0.2	0.01	<DL	<DL	<DL	<DL
Acidic phase	ND	ND	93.9	1.32	109.7	2.49	83.2	3.73	92.3	0.49	98.2	1.07
Aqueous alkaline phase	8.4	0.02	11.4	0.16	<DL	<DL	2.2	0.10	<DL	<DL	3.3	0.04
DPA/DCM	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	88.2	0.96
DCM	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Acid aqueous phase	92.8	0.22	68.5	0.96	90.2	2.05	77.3	3.47	90.3	0.47	87.1	0.95
Final dilution; Acetone/methanol eluent[b]	72.5	0.17	57.3	0.80	85.0	1.93	68.6	3.07	75.2	0.40	73.6	0.80
Ion chromatographic analysis[c]	**74.2**	**0.18**	**51.3**	**0.72**	**90.7**	**2.06**	**72.9**	**3.27**	**82.7**	**0.43**	**80.0**	**0.87**

[a]Duplicate samples were analyzed; data on subsample-1 are presented here unless otherwise indicated. [b]Final extract; analyzed by IC according to method 286. [c]Ion Chromatographic results from analysis of final extracts for mepiquat chloride. [d]Data from subsample-2 presented.

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Table 11. Recoveries of mepiquat chloride from fortified poultry and ruminant tissues as determined by BASF method 286.

Substrate	MRID	Fortification Level	Percent Recovery
Poultry:			
Eggs	42394302[a]	0.05	65.8-99.2 (4)[b]
		5.0	59.1, 59.3, 66.2-76.9 (5)
	42394304	1.0	72.3
Muscle	42394302	0.05	90.5-101.3 (5)
		5.0	74.8-82.8 (5)
	42394304	0.4	60.3
Liver	42394302	0.05	82.7-90.3 (5)
		5.0	86.2-89.5 (5)
	42394304	1.0	90.8
Skin	42394302	0.05	98.2-108.0 (4)
		5.0	71.3-79.6 (5)
	42394304	1.0	86.9
Fat	42394302	0.05	91.4-108.1 (5)
		5.0	73.3-84.8 (5)
	42394304	1.0	84.7
Ruminant: (Cow)			
Milk	42394302	0.05	76.2-90.1 (5)
		5.0	67.7-83.7 (5)
	42394303	0.3	69.7
Muscle	42394302	0.05	68.8-121.7 (5)
		5.0	77.5-90.0 (5)
	42394303	5.0	81.1
Fat	42394302	0.05	82.6-110.0 (5)
		5.0	74.0-80.0 (5)
	42394303	0.3	81.4
Liver	42394302	0.05	89.2-98.4 (5)
		5.0	77.9-88.6 (5)
	42394303	20.0	78.5
Kidney	42394302	0.05	87.6-100.6 (5)
		5.0	65.3-68.4 (5)
	42394303	20.0	85.0

[a]Recoveries corrected for residues in controls (all <0.01 ppm). [b]Number of samples analyzed.

References

Citations for the MRID documents referenced in this review are presented below. Submissions reviewed in this document are indicated by shaded type.

41585201 Kohl, W. (1989) The Metabolism of [Carbon 14]-Mepiquat Chloride in Laying Hens: BASF Registration Document No.: 89/0312. Unpublished prepared by BASF AG, Agricultural Research and Development. 112 p.

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41585203 Cheng, T. (1988) Biokineticss and Metabolism Study of [Carbon 14]-BAS 083 W in Laying Hens: BASF Registration Document No.: 89/5021. Unpublished study prepared by Hazleton Laboratories America. 73 p.

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41585206 Cheng, T. (1988) Biokinetics and Metabolism Study of [Carbon 14]-BAS 083 W in Lactating Goats: BASF Registration Document No.: 89/5022. Unpublished study prepared by Hazleton Laboratories America. 69 p.

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Agency Memoranda

CBRS No.: 10229
Subject: Response to the Mepiquat Chloride Phase IV Review: Cotton Metabolism
From: R. Perfetti
Dated: 3/5/93
MRID(s): 42330804

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