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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: **Oxyfluorfen.** Analytical Method for Meat/Milk/Eggs and Response to CBRS Review of Ruminant Metabolism Study. MRID #43307502 43346401 43307503 and 43317701 DP Barcode D207134 CBRS #14321 and 14323

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THRU: Edward Zager, Chief
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TO: Mark Wilhite, PM Team 53
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The Phase 4 Review for oxyfluorfen (S.Funk, 3/16/91) required the registrant to submit data collection and regulatory analytical method validation data for the determination of oxyfluorfen and its diphenyl ether metabolites in/on eggs and liver. It was also noted that any new regulatory methods submitted will require an independent method validation.

In response, Rohm and Haas has submitted analytical enforcement methods for meat, milk and eggs (MRIDs #43307502, 43346401, and 43307503). The structure of oxyfluorfen and its major isomers are presented in Table 1.

Tolerances are established for residues of the herbicide oxyfluorfen [2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene] and its metabolites containing the diphenyl ether linkage in or on various commodities including, but not limited to: fat, meat and mbyp of sheep, poultry, horses, hogs, goats and cattle at 0.05 ppm; eggs at 0.05 ppm; milk at 0.05 ppm [40 CFR §180.381 (a)]. A food additive tolerance of 0.25 ppm is established for

residues of oxyfluorfen and its metabolites containing the diphenyl ether linkage in or on the processed commodities cottonseed oil, mint oil (peppermint and spearmint) and soybean oil as a result of application of the herbicide to the growing crops [40 CFR §185.4600].

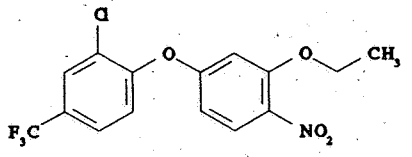
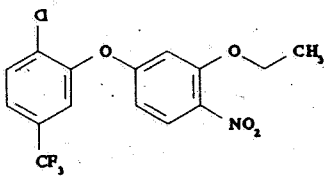
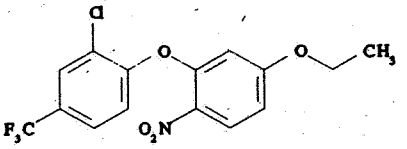
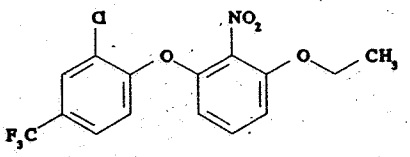
Recommendations

The submitted analytical enforcement meat/milk/egg methods are not adequate but are upgradeable by responding to Conclusions 1-27. No new data need to be generated to respond to most of the conclusions (except for radiovalidation of the method using egg samples from the metabolism study and development of a GC/MS confirmatory method). The majority of the deficiencies involve clarifications to the method write-ups that will result in more rugged methods or corrections necessitated by Branch policies. The registrant is reminded that following the requested modifications to the methods, the methods must undergo independent laboratory validation (ILV) as described in PR Notice 88-5 (July 15, 1988). Following the ILV the Agency will conduct its method tryout.

The response to the previous ruminant metabolism study adequately addresses all deficiencies, except for the storage stability requirements. The registrant stated that a storage stability study is currently underway. Until these studies are submitted, reviewed, and found to be adequate, this deficiency remains.

Note to PM: Please provide the registrant with a complete copy of this review.

Table 1. Oxyfluorfen and its isomers.

Common Name Chemical Name	Structure
Oxyfluorfen 2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene	
RH-2382 2-chloro-1-(3-ethoxy-4-nitrophenoxy)-5-(trifluoromethyl)benzene	
RH-4672 2-chloro-1-(3-ethoxy-6-nitrophenoxy)-4-(trifluoromethyl)benzene	
RH-0671 2-chloro-1-(3-ethoxy-2-nitrophenoxy)-4-(trifluoromethyl)benzene	

Conclusions

Meat and Fat Analytical Method (MRID #43307502)

1. Insufficient documentation concerning the standards was provided. The registrant should provide the dates of synthesis, analysis, and expiration dates. In the instructions for preparation of standards, the registrant failed to note that when weighing 10 mg of each analytical standard, corrections for percent purity of each compound should be made.

2. In the extraction instructions, for clarity, the registrant should insert "For meat (muscle, kidney, and liver)". This approach is consistent with the instructions provided for the partitioning steps.

3. The registrant claims that final sample extracts (concentrated samples obtained after cleanup by column chromatography) can be stored for 1-2 weeks prior to GLC analysis. Data must be provided to support this claim and storage conditions should be described.

4. CBRS notes that the Restek columns used for analysis and confirmation are of generally similar polarity (Rtx 200 = trifluoropropylmethyl and Rtx-50 = 50% methyl/50%phenyl polysiloxane respectively) and both GLC techniques use ECD. CBRS would prefer if GC/MS with selected ion monitoring be used for confirmation. If the registrant does not develop an acceptable alternative confirmatory method, interference testing will be necessary.

5. Standard Curves
5.a. CBRS notes that there appears to be a typographical error on page 13 of the study. No units are stated following the list of concentrations for construction of the standard curve, though obviously the units are ug/mL. This error must be corrected.

5.b. CBRS concludes that the registrant should not use quadratic regression to construct standard curves. CBRS prefers that with ECD, linear regression analysis be used for construction of standard curves. For the representative data provided for standards, linear regression analysis provides a good fit as measured by the correlation coefficient (r ranged from 0.9988 to 0.9991 for all analyte/column combinations). Based on these results there is no need to use a quadratic fit for the standard curves. The method write-up should be modified to reflect use of a linear fit.

6. Quantitation - For clarity and consistency, in Equation 1, the registrant should change "Total volume (mL)" to "Final sample volume (mL)".

7. Fortification - CBRS prefers that when analyses are corrected for matrix effects (background corrections) both corrected and uncorrected results be reported. The registrant should provide this information.

8. Limit of Detection/Limit of Quantitation - The registrant stated that the limit of quantitation (LOQ) will be determined by additional actual fortifications. The registrant claims that the tentative LOQ appears to be 0.010 ppm and that the limit of detection (LOD) appears to be 0.003 ppm for all analytes. CBRS concludes that sufficient data has been presented to support 0.010 ppm as the LOQ for oxyfluorfen and its isomers RH-2382, RH-4672, and RH-0671, in muscle, fat, liver, and kidney.

9. Fortifications - The registrant did not supply an example calculation for one of the fortified samples. An example calculation, including all supporting data (chromatograms, peak height measurements, standard curve used, etc.) must be provided.

10. Results -
10.a. Muscle - Adequate recoveries were obtained for cow and hen muscle samples fortified at 0.010 - 1.00 ppm.

10.b. Fat - With the exception of two cow fat samples, recoveries for cow and hen fat were acceptable. In light of the other cow fat and the hen fat results, it appears that there may have been an error in fortification of samples O07A10 and O07A11. The registrant should reexamine all data pertinent to these sample to determine if an error in fortification was made or provide an alternative explanation of these results. CBRS also notes that recoveries for concurrent fortifications for cow fat analyzed in conjunction with the radiovalidation gave acceptable recoveries (78.9 - 112 % for all analytes fortified at 0.02, 0.05, and 0.10 ppm).

10.c. Liver - Adequate recoveries were obtained for cow and hen liver samples fortified at 0.010 - 1.00 ppm.

10.d Kidney - Adequate recoveries were obtained for cow kidney samples fortified at 0.010 - 1.00 ppm.

11. Radiovalidation -

11.a. The registrant did not identify which samples from the metabolism study were analyzed. This is a deficiency. Because different levels of oxyfluorfen were found for the various radiolabels, the registrant should identify exactly which samples from the metabolism study were analyzed by the analytical method.

12.b. For the oxyfluorfen isomers, the registrant reported all results as <0.003 ppm for both the metabolism and analytical methods, with the exception of 0.032 ppm of RH-2382 found in hen breast muscle by the analytical method. The chlorophenyl and nitrophenyl ^{14}C-labeled oxyfluorfen used in the metabolism studies (MRID #42670601 - ruminant and #42634701 - poultry) had radiochemical purities of 100% and 95% respectively, that is, none of the isomers were present to a significant extent. In light of this fact, the registrant should provide a possible explanation for RH-2382 being found in hen breast muscle.

12.c. Until the registrant provides additional data supporting the proposed LOD of 0.003 ppm for the analytical method, the non-detectable results reported by the registrant as <0.003 ppm in Table 8 of the study (page 23) should be changed to <0.010 ppm.

12.d. Although the registrant indicated that results should not be corrected for percent recovery of concurrent fortifications (as shown by Equation 1 in the method), the results were actually corrected. Uncorrected results were calculated by this reviewer.

12.e. With the exception of hen liver, results obtained from the metabolism study and the analytical method were comparable. In hen liver the analytical method only recovered approximately 50% of the oxyfluorfen that was quantitated in the metabolism study.

Milk Analytical Method (MRID #43346401)

13. Reference Standards - The same reference standards used in the meat analytical method study were used for this study. Additional information concerning the standards must be supplied as noted above in Conclusion 1 for the meat study.

14. Extraction - CBRS notes that there appears to be a typographical error on page 8 of the study. For the hexane partition of the aqueous phase, instructions should most likely read, "Partition again as described above", instead of as "desired". The registrant must modify this section of the procedure appropriately.

15. The registrant did not state which GLC column was used for analysis and which was used for confirmation. The comments noted in the review of the meat method concerning the similar polarities of the columns are applicable here too (Rtx-2300 = 90% biscyanopropyl/10% phenylcyanopropyl polysiloxane). Therefore, unless a confirmatory method that differs more from the analytical method is developed (preferably GC/MS with selected ion monitoring), interference testing for other active ingredients with tolerances for milk will be required.

16. Standard Preparation - CBRS concludes that the registrant must rewrite the instructions for standard preparation with more detail, as was done in the meat and egg methods.

17. Standard Curves - As for the meat method, CBRS concludes that the registrant should use linear regression analysis for construction of standard curves. The method write-up must be modified to use linear fits for standard curves. Although representative standard curves were provided for each analyte/column combination, the raw data (peak heights and ug/mL) used to construct the curves were not provided. This is a deficiency.

18. Quantitation - CBRS notes that Branch policy does not allow for correcting residue results for percent recovery of concurrent fortifications. Equation 1 must be modified to remove average percent recovery from the denominator. Additionally, for clarity and consistency (see Eq. 2 below) the registrant should change "Total volume (mL)" to "Final sample volume (mL)". For fortifications, CBRS prefers that when analyses are corrected for matrix effects (background corrections) both corrected and uncorrected results be reported. The registrant should report results this way.

19. Fortifications -

19.a. The registrant proposed 0.010 ppm as the tentative limit of quantitation (LOQ). Based on the available data, an LOQ of 0.010 ppm appears to be appropriate for residues of oxyfluorfen in milk. For residues of the isomers RH-2382, RH4672, and RH-0671 in milk, 0.020 ppm appears to be an appropriate LOQ. CBRS reserves final judgement on this issue until receipt of sample identification information requested in Conclusion 19.c.

19.b. CBRS notes that the registrant did not specify which GLC column was used to generate the data presented in the summary table. This is a deficiency. Additionally, both uncorrected and background corrected results must be presented.

19.c. CBRS also notes that from the way the data summary was presented it is unclear if three separate samples were analyzed at the indicated fortification levels or if the same sample was analyzed three times. The registrant should provide a more detailed data summary table, including sample identification codes, dates of extractions, dates of analysis, and any other pertinent information.

19.d. The registrant did not supply an example calculation for one of the fortified samples. An example calculation, including all supporting data (chromatograms, peak height measurements, standard curve used, etc.) must be provided.

20. Radiovalidation - The registrant did not identify which samples from the metabolism study were analyzed. This is a deficiency.

20.a. The chlorophenyl and nitrophenyl ^{14}C-labeled oxyfluorfen used in the metabolism study had radiochemical purities of 100% and 95% respectively, that is, none of the isomers were present to a significant extent. Until the registrant can provide additional data supporting the proposed LOD of 0.003 ppm for the analytical method, the non-detectable results (reported as <0.003 ppm) listed in Table should be changed to <0.020 ppm.

20.b. The results reported for the analytical method were corrected for percent recovery. The registrant should report uncorrected results. It appears that similar results are obtained from the metabolism study and analytical method for oxyfluorfen residues in milk, however, uncorrected results are needed before definitive conclusions can be made.

Egg Analytical Method (MRID #43307503)

21. No information concerning the purity or lot numbers of the reference standards was provided. This is a deficiency. Additionally, information concerning the standards must be supplied as noted above in Conclusion 1 of the meat study.

22. Concerning the confirmatory method, the comments noted in the review of the meat method are applicable here too. Unless a confirmatory method that differs more from the analytical method is developed (preferably GC/MS with selected ion monitoring), interference testing for other active ingredients with tolerances in/on eggs will be required.

23. CBRS notes that Branch policy does not allow for correcting residue results for percent recovery in fortification samples. Equation 2 of the study must be modified to remove average percent recovery from the denominator. Additionally, for clarity the registrant should change "Total volume (mL)" to "Final sample volume (mL)".

24. When determining percent recovery for fortification samples, CBRS prefers that if analyses are corrected for matrix effects (background corrections) both corrected and uncorrected results be reported. The registrant should provide this information.

25. Standard Curves - Although representative standard curves were provided for each analyte/column combination, the raw data (peak heights and ug/mL) used to construct the curves were not provided. This is a deficiency. All raw data used to construct a representative standard curve should be provided (one set of data for each GLC column). As noted above, CBRS prefers that linear regression analysis be used instead of quadratic regression. The method write-up should be modified to reflect this change.

26. Fortification Results -
26.a. The registrant should provide a more detailed data summary table, including sample identification codes, dates of extractions, dates of analysis, and any other pertinent information. The registrant did not supply an example calculation for one of the fortified samples. This is a deficiency. An example calculation, including all supporting data (chromatograms, peak height measurements, standard curve used, etc.) must be provided.

26.b. CBRS notes that for all analyte fortifications $\geq$ 0.010 ppm, with two exceptions (noted in shading in the data summary tables) recoveries are generally in the range considered acceptable by the Agency (70 - 120%). The submitted data support a 0.010 ppm LOQ for oxyfluorfen and its isomers RH-2382, RH-4672, and RH-0671 in eggs.

27. Radiovalidation - The method was not radiovalidated using egg samples from the metabolism study. This is a deficiency. Eggs from the poultry metabolism study contained 1.037 and 1.026 ppm ^{14}C-oxyfluorfen (chlorophenyl and nitrophenyl ring labeled respectively). The registrant must submit radiovalidation data for this method.

Response to Review of Ruminant Metabolism Study

28. The registrant has adequately addressed the deficiency concerning an explanation for the sudden increase in radioactive residues in the day 4 milk sample.

29. The storage stability deficiency remains pending submission and review of storage stability studies currently underway.

30. The requirement for radiovalidation of the analytical method using meat/milk samples from the metabolism study has been fulfilled.

31. The submitted supplemental study (MRID #43317701) for characterization of radioactive residues in liver is adequate. The predominant liver metabolites identified were amino-oxyfluorfen (and its conjugates) and amino-hydroxy-oxyfluorfen (and its conjugates).

Detailed Considerations

Note: Modifications to the method write up made by this reviewer are indicated in shaded type throughout this review. These modifications were made to improve the clarity of the write-up and should be incorporated by the registrant.

Meat and Fat Analytical Method (MRID #43307502)

Reference Standards - Reference standards consisted of oxyfluorfen (RH-2915) and three isomers (RH-2382, RH4672, and RH-0671). Lot numbers and purity of the standards were provided. Purity ranged form 96.9% to 99.9%.

Insufficient documentation concerning the standards was provided. The registrant should provide the dates of synthesis, analysis, and expiration date.

Sample Preparation - Instructions for sample preparation are as follows:

> "Muscle, kidney, liver, and fat are homogenized with dry ice in a Hobart processor. The dry ice is allowed to sublime overnight in a freezer. Samples are maintained frozen prior to analysis."

Extraction - The extraction procedure is described as follows:

> "Weigh a homogenized 5.0 g sample into a 250 ml centrifuge bottle. For meat (muscle, kidney, and liver): Add 100 mL of acetonitrile and homogenize with a Tissumizer at medium speed for 1-2 minutes. Centrifuge the extraction mixture at 5000 rpm for 20 minutes at 4 C. Decant the supernatant into a 500 mL separatory funnel. Add another 100 mL of ACN to the centrifuge bottle containing the tissue pellet, then mix well and centrifuge again. Decant the supernatant into the 500 mL separatory funnel with the first ACN extract. For fat, add 100 mL of hexane and place the centrifuge bottle in a warm water bath, at about 70 C for 15 minutes, then homogenize with a Tissuemizer at medium speed for 1-2 minutes. Centrifuge the extraction mixture at 5000 rpm for 20 minutes at 4 C. Decant the supernatant into a 500 mL separatory funnel and wash the centrifuge bottle with 2 x 5 mL hexane and add the washes to the same separatory funnel."

For clarity, the registrant should insert "For meat (muscle, kidney, and liver)" as shown in shaded type above. This approach is consistent with the instructions provided for the partitioning steps (see Partition 1 below).

The registrant noted that instead of centrifugation, samples may be filtered under suction with a vacuum pump (about 50 mm Hg) through a Whatman #41 filter paper that has been prewashed with ACN and dried.

Partitioning -Two partitioning steps are used as follows:

> "Partition 1 - For muscle, liver, and kidney, add 200 mL of petroleum ether to the 500 mL separatory funnel containing the 200 mL ACN extract, shake for 20 seconds and vent into a fume hood. Collect the ACN extract into a 500 mL round bottom flask. Discard the petroleum ether layer to waste. Evaporate the ACN extract to 50-80 mL by rotary evaporator at 50-70 C under diminished pressure.
>
> For fat, add 100 mL ACN to the 500 mL separatory funnel containing 110 mL hexane extract, shake for 1 min and vent in a fume hood. Collect the ACN extract into a 500 mL round bottom flask. Partition

the hexane layer with another 100 mL ACN and combine the second extract with the first extract in the same 500 mL round bottom flask. Discard the hexane layer to waste. Transfer the 200 mL ACN extract into another 500 mL separatory funnel. Add 200 mL petroleum ether, shake for 20 seconds and vent in a fume hood. Collect the ACN extract in to a 500 ml round bottom flask. Discard the petroleum ether layer to waste. Evaporate the ACN to 50-80 mL by rotary evaporator at 50-70 C under diminished pressure, and quantitatively transfer to a 500 mL separatory funnel.

Partition 2 - At this point, meat and fat samples are handled in a similar manner. Add 100 mL of petroleum ether to the 500 mL separatory funnel containing the 50-80 mL ACN extract. Add 10 mL of saturated NaCl solution and 200 mL of water into the separatory funnel. Shake vigorously for 2 minutes and vent into a fume hood. Drain the ACN/water lower phase and reserve for further partitioning, the collect the petroleum ether phase in a 24/40 ST 500 mL round bottom flask. For meat (muscle, liver, and kidney), partition the reserved ACN/water phase with another 100 mL of petroleum ether. For fat, partition the reserved ACN/water phase with another 50 mL of petroleum ether twice. Drain the lower ACN/water layer to waste. Combine all petroleum ether fractions in the same 500 mL round bottom flask. Evaporate gently to approximately 5 mL of petroleum ether by rotary evaporator at 40 C under diminished pressure."

Column Cleanup - Silica gel or Florisil column chromatography are used to clean up extracts as follows:

"Silica Gel Chromatography (Muscle and Kidney) - Activate the silica gel, mesh 60-100, by heating for 24 hours at 200 C. Remove from the oven and store in tightly capped jars in a desiccator. Pack a 250 mm x 16.0 mm ID glass column plugged with cotton with 20 mL of the activated silica gel. Top the column with 10-15 g (2-3 cm of column height) of anhydrous granular sodium sulfate.

Add the 5 mL of the remaining petroleum ether extract of meat to the column and elute to the top of the silica gel bed. Add 30 mL of petroleum ether to the 500 mL round bottom flask, transfer to the column, and elute to the top of the silica gel bed. Add 15 mL of 80/20 (v/v) petroleum ether/ethyl ether to the 500 mL round bottom flask, transfer to the column, and elute to the top of the silica gel bed. Discard all washes to this point.

Elute the oxyfluorfen and the RH-2382/RH-4672/RH-0671 isomers by adding 75 mL of 60/40 (v/v) petroleum ether/ethyl ether to the 500 mL round bottom flask, transfer to the column, and elute to the top of the silica gel bed. Collect the eluate in a 100 mL round bottom flask with a 24/40 ST. Evaporate to dryness at 45 C under diminished pressure. Redissolve in 5 mL of toluene. The sample is now ready for GLC quantitation. The final sample extract can be stored in the round bottom flask for a 1-2 week period before injection.

Florisil Column Chromatography (Fat and Liver) - Activate the Florisil, mesh 60-100, by heating for 24 hours at 150 C maximum. Remove from the oven and store in tightly capped jars in a desiccator. Pack a 250 mm x 16.0 mm ID glass column plugged with cotton with 20 mL of the activated Florisil. Top the column with 10-15 g (2-3 cm of column height) of anhydrous granular sodium sulfate.

Add the 5 mL of the remaining petroleum ether extract of fat or liver to the column and elute to the top of the Florisil bed. Add 30 mL of petroleum ether to the 500 mL round bottom flask, transfer to the column, and elute to the top of the Florisil bed. Add 20 mL of 80/20 (v/v) petroleum ether/ethyl ether to the 500 mL round bottom flask, transfer to the column, and elute to the top of the silica gel bed. Discard all washes to this point.

Elute the oxyfluorfen and the RH-2382/RH-4672/RH-0671 isomers by adding 75 mL of 10/90 (v/v) petroleum ether/ethyl ether to the 500 mL round bottom flask, transfer to the column, and elute to the

top of the silica gel bed. Collect the eluate in a 100 mL round bottom flask with a 24/40 ST. Add 2-3 drops of octanol into the flask. Evaporate to dryness at 45 C under diminished pressure. Redissolve in 5 mL of toluene. The sample is now ready for GLC quantitation. The final sample extract can be stored in the round bottom flask for a 1-2 week period before injection."

CBRS notes that the registrant claims that final sample extracts can be stored for 1-2 weeks prior to GLC analysis. Data must be provided to support this claim.

GLC Chromatography - A Varian 3500 Capillary GC equipped with a Varian model 8100 Auto Sampler and a Capillary Thermionic Detector was used. Data were obtained with a HP 300 Data Acquisition System with HP Extrachrom Software. Data were processed with Nelson Analytical Software.

Two analytic GLC columns can be used. Column 1 is the primary column, and column 2 is the confirmatory column.

Column 1:

-Analytical Column - Restex Rtx-200, 0.32 mm ID, 60 m, 1.0 u film, Catalog #15057
-Gaurd Column - Restex deactivated uncoated fused silica gel, 5 m, 0.53 mm ID, Catalog #10045
-Column Connection - Supelco Glass Seal Connector, Catalog #2-0479
-Gas Flows - Nitrogen (makeup) 40 mL/min, Nitrogen (column) 3.5 mL/min
-Temperatures - Injector 265 C, Detection 300 C, Column 215 C, Initial Hold 1.0 min
-Column Program - Final temp 250 C, Rate 5.0 C/min, Hold 17.0 min, Total time 25 min
-Electron Capture Detection - Attenuation 32, Range 10
-Retention Times - RH-0671 14.5 min; RH-2382 15.1 min; RH-2915 16.3 min; RH-4672 16.7 min
-Injection Volume - 1uL

Column 2:

-Analytical Column - Restex Rtx-50 megabore Column, 0.32 mm ID, 60 m, 1.0 u film, Catalog #10557
-Guard Column - Restex deactivated uncoated fused silica gel, 5 m, 0.53 mm ID, Catalog #10045
-Column Connection - Supelco Glass Seal Connector, Catalog #2-0479
-Gas Flows - Nitrogen (makeup) 40 mL/min, Nitrogen (column) 3.5 mL/min
-Temperatures - Injector 265 C, Detection 300 C, Column 215 C, Initial Hold 3.0 min
-Column Program - Final temp 250 C, Rate 5.0 C/min, Hold 25.0 min, Total time 33 min
-Electron Capture Detection - Attenuation 32, Range 10
-Retention Times - RH-0671 21.4 min; RH-2382 20.0 min; RH-2915 22.4 min; RH-4672 23.0 min
-Injection Volume - 1 uL

CBRS notes that the Restek columns used for analysis and confirmation are of generally similar polarity (Rtx 200 = trifluoropropylmethyl and Rtx-50 = 50% methyl/50%phenyl polysiloxane respectively) and both GLC techniques use ECD. CBRS would prefer if GC/MS with selected ion monitoring be used for confirmation. If the registrant does not develop an acceptable alternative confirmatory method, interference testing will be necessary.

Preparation of Standard Curves - Instructions for preparation of standards and standard curves follow:

> "Standard solutions of RH2915/RH-0671/RH2382/RH-4672 in toluene are prepared (by serial dilution) in the concentration range 0.005 ug/mL - 0.20 ug/mL. Prepare a 200 ppm standard stock solution by weighing 20 mg of each analyte into individual 100 mL volumetric flask. Then, make up a 10 ppm stock solution by adding 5.0 mL of each 200 ppm solution into a 100 mL volumetric flask.
>
> Prepare standard solutions of 0.005, 0.01, 0.02, 0.05, 0.10, and 0.15 ug/mL by diluting the 10 ppm stock solution. One microliter of each standard solution is injected and the resulting standard curve is constructed by plotting peak heights measured versus concentration (ug/mL). The standard curves are constructed by quadratic regression within the concentration range. Standard curves are prepared for each analysis day."

CBRS notes that there appears to be a typographical error on page 13 of the study. No units are stated following the list of concentrations for construction of the standard curve, though obviously the units are ug/mL (as noted by this reviewer in the shaded text of the preceding paragraph). This error must be corrected.

CBRS concludes that the registrant should use linear regression analysis for construction of the standard curve. See additional discussion under Results.

Quantitation One microliter of the sample is injected into the GLC. If necessary, the sample is diluted to an appropriate volume to give a response within the standard curve range. The peak height is measured and the concentration is determined from the standard curve. The registrant stated that the concentration is then determined as follows:

$$\text{Eq. 1} \quad \frac{\text{Total volume (mL)} \times \text{Concentration (ug/mL)} \times 100}{\text{Sample weight (g)}} = \text{ppm}$$

For clarity and consistency (see Eq. 2 below) the registrant should change "Total volume (mL)" to "Final sample volume (mL)".

Fortification The registrant states,

> "For samples fortified with known amounts of RH-2915/RH-0671/ RH2382/RH-4072 prior to extraction, measure peak height, determine the ug/mL from the standard curve, correct for any background in the control sample, and calculate percent recovery from equation 2.

$$\text{Eq. 2} \quad \frac{[(\text{ug/mL}) \text{ found} \times \text{Final sample volume (mL)} - \text{ug control}] \times 100}{\text{ug added}} = \text{\% Recovery"}$$

CBRS prefers that when analyses are corrected for matrix effects (background corrections) both corrected and uncorrected results be reported (see below).

Results

The registrant stated that the limit of quantitation (LOQ) will be determined by additional actual fortifications. The registrant claims that the tentative LOQ appears to be 0.010 ppm and that the limit of detection (LOD) appears to be 0.003 ppm for all analytes.

CBRS recommends that the registrant provide all pertinent data in support of their tentative LOD and LOQ when they respond to the deficiencies raised in this review.

Standard Curves - For both columns, representative standard chromatograms (including printouts of retention times and peak heights) and standard (calibration) curves were provided. All calibration curves were constructed using quadratic fitting of the data. As noted above, for linear response detectors, CBRS prefers that linear regression analysis be used instead of quadratic regression. For the representative data provided for standards, linear regression analysis provides a good fit as measured by the correlation coefficient (r). Correlation coefficients calculated by this reviewer are presented in Table 2.

Table 2. Correlation coefficients obtained from linear regression analysis of standard calibration curves. Raw data were extracted from Figures 1-5 for Rtx-200 column and Figures 35-39 for Rtx-50 column. Correlation coefficients were obtained from regressions analysis of linear fits of concentration (ug/mL) versus peak heights. Concentration of standards were 0.005, 0.010, 0.050, 0.100, and 0.0150 ug/mL.

Column	Correlation Coefficients Calculated for Various Analytes			
	RH-0671	RH-2382	RH-2915 (oxyfluorfen)	RH-4672
Rtx-200	0.9991	0.9988	0.9989	0.9988
Rtx-50	0.9989	0.9988	0.9988	0.9988

Fortifications

Muscle - For cow muscle samples fortified at 0.010 - 1.00 ppm (n = 16) recoveries for oxyfluorfen and its isomers ranged from 71.8 - 106%. For hen muscle samples fortified at 0.010 - 1.00 ppm (n = 16) recoveries for oxyfluorfen and its isomers ranged from 59.9 - 92.7%.

Fat - For cow fat samples fortified at 0.010 - 1.00 ppm, with the exception of two samples, recoveries for oxyfluorfen and its isomers ranged from 94.8 - 114%. For sample O07A10, fortified with RH-0671, RH-2382, oxyfluorfen, and RH-4672 at 0.010 ppm recoveries were 155, 159, 156, and 151% respectively. For sample O07A11, fortified with RH-0671, RH-2382, oxyfluorfen, and RH-4672 at 0.050 ppm recoveries were 131, 136, 140, and 138% respectively.

For hen fat samples fortified at 0.010 - 1.00 ppm, recoveries for oxyfluorfen and its isomers ranged from 86.3 - 116%.

In light of the other cow fat and the hen fat results, it appears that there may have been an error in fortification of samples O07A10 and O07A11. The registrant should reexamine all data pertinent to these sample to determine if an error in fortification was made or provide an alternative explanation of these results. CBRS also notes that recoveries for concurrent fortifications for cow fat analyzed in conjunction with the radiovalidation gave acceptable recoveries (78.9 - 112 % for all analytes fortified at 0.02, 0.05, and 0.10 ppm).

Liver - For cow liver samples fortified at 0.010 - 1.00 ppm, recoveries for oxyfluorfen and its isomers ranged from 60.0 - 105%. For hen liver samples fortified at 0.010 - 1.00 ppm, recoveries for oxyfluorfen and its isomers ranged from 66.9 - 108%.

Kidney - For cow kidney samples fortified at 0.010 - 1.00 ppm, recoveries for oxyfluorfen and its isomers ranged from 66.7 - 104%.

Radiovalidation - Table 3 presents results of the method radiovalidation using samples obtained from the metabolism study. The registrant did not identify which samples from the metabolism study were analyzed. This is a deficiency. Because different levels of oxyfluorfen were found for the various radiolabels, the registrant should identify exactly which samples from the metabolism study were analyzed by the analytical method.

For the oxyfluorfen isomers, the registrant reported all results as <0.003 ppm for both the metabolism and analytical methods, with the exception of 0.032 ppm of RH-2382 found in hen breast muscle by the analytical method. The chlorophenyl and nitrophenyl ^{14}C-labeled oxyfluorfen used in the metabolism studies (MRID #42670601 - ruminant and #42634701 - poultry) had radiochemical purities of 100% and 95% respectively, that is, none of the isomers were present to a significant extent. In light of this fact the registrant should explain how RH-2382 was found in hen breast muscle.

Until the registrant provides additional data supporting the proposed LOD of 0.003 ppm for the analytical method, the non-detectable results reported by the registrant as <0.003 ppm in Table 8 of the study (page 23) should be changed to <0.01 ppm.

Table 3. Comparison of metabolism study and meat analytical method results.

Sample	ppm Oxyfluorfen Found		
	Metabolism	Residue Uncorrected	Average Residue Corrected for % Recovery
Hen Breast Muscle	0.145	0.187, 0.184	0.217
Hen Thigh Muscle	1.029	0.093, 0.937	1.074
Hen Fat	13.769	11.20, 11.00, 10.70	12.5
Hen Liver	0.776	0.291, 0.233	0.346

Sample	ppm Oxyfluorfen Found		
	Metabolism	Residue Uncorrected	Average Residue Corrected for % Recovery
Goat Muscle	0.022	0.022, 0.024	0.025
Goat Fat	0.511	0.425, 0.454	0.485
Goat Liver	<0.003	<0.003, <0.003	<0.003
Goat Kidney	0.003	<0.003, <0.003	<0.003

Milk Analytical Method (MRID #43346401)

Reference Standards - The same reference standards used in the meat analytical method study were used for this study. Additional information concerning the standards must be supplied as noted above for the meat study.

Extraction - Milk is homogenized with a Polytron homogenizer for 2 minutes immediately before sampling. The registrant described the extraction procedure as follows:

> "Weigh a homogenized 5.0 g sample of milk into a glass sample vial. Quantitatively transfer sample to a 500 mL separatory funnel containing 45 mL of 10% NaCl aqueous solution (10g NaCl in 100 ml HPLC water). Wash the glass sample vial twice with 2.5 mL of the 10% NaCl solution (into the 500 mL separatory funnel). The 500 mL separatory funnel now contains the 5.0 g sample and 50 mL of 10% NaCl aqueous solution. Add to the separatory funnel 150 mL of a 1:1 (v/v) hexane/acetone solution. Shake the separatory funnel gently by hand for 5 seconds and vent in a fume hood. Repeat until no further pressure build up occurs.
>
> Transfer the lower aqueous phase into a second 500 mL separatory funnel containing 150 mL of hexane. Partition again exactly as desired. Discard the lower aqueous layer. Combine both hexane layers in the same 500 mL round bottom flask by passing the layers through a bed of 100 g anhydrous sodium sulfate contained in a powder funnel (100 mm diameter) plugged with cotton. Evaporate to dryness by rotary evaporator at 45 C under diminished pressure."

CBRS notes that there appears to be a typographical error on page 8 of the study. For the hexane partition of the aqueous phase, instructions should most likely read, "Partition again as described above", instead of as "desired". The registrant must modify this section of the procedure appropriately.

Florisil Column Chromatography - Instructions are as follows:

> "Activate the Florisil, mesh 60 -100, by heating for 24 hours at 200 C. Remove from the oven and store in tightly capped jars in a desiccator. Pack a 250 mm x 16.0 mm ID glass column plugged with cotton with 20 mL of the activated Florisil. Top the column with 1 g of anhydrous sodium sulfate.

Redissolve the residue from the hexane partition in 15 mL of toluene. Add to the column and elute to the top of the Florisil gel bed. Add 30 mL of toluene to the 500 mL round bottom flask, transfer to the column and elute to the top of the Florisil. Add 10 mL of 90/10 (v/v) toluene/methanol to the 500 mL round bottom flask, transfer to the column, and elute to the top of the florisil bed. Discard all washes to this point. Elute the oxyfluorfen and the RH-2382/RH-4672/RH-0671 isomers by adding 25 mL of 80/20 (v/v) toluene/methanol to the 500 mL round bottom flask, transferring to column, and eluting to the top of the Florisil bed. Collect the eluate in a 100 mL round bottom flask with a 24/40 ST. Evaporate to dryness at 45 C under diminished pressure. Redissolve the toluene in 5 mL of toluene. The sample is now ready for GLC quantitation."

GLC Chromatography - A Varian 3500 Capillary GC equipped with a Varian model 8100 Auto Sampler and a Capillary Thermionic Detector was used. Data were obtained with a HP 300 Data Acquisition System with HP Extrachrom Software. Data were processed with Nelson Analytical Software.

Two primary analytic GLC columns can be used:

Column 1:

-Analytical Column - Restex Rtx-2330 megabore Column, 0.32 mm ID, 60 m, 0.20 u film, Catalog #10727
-Guard Column - Restex deactivated uncoated fused silica gel, 5 m, 0.53 mm ID, Catalog #10045
-Column Connection - Supelco Glass Seal Connector, Catalog #2-0479
-Gas Flows - Nitrogen (makeup) 20 mL/min, Nitrogen (column) 4.1 mL/min
-Temperatures - Injector 250 C, Detection 270 C, Column 215 C, Initial Hold 3.0 min
-Column Program - Final temp 230 C, Rate 5.0 C/min, Hold 15.0 min, Total time 21 min
-Electron Capture Detection - Attenuation 32, Range 10
-Retention Times - RH-0671 12.1 min; RH-2382 12.8 min; RH-2915 14.4 min; RH-4672 14.8 min

Column 2:

-Analytical Column - Restex Rtx-200, 0.32 mm ID, 60 m, 1.0 u film, Catalog #15057
-Gaurd Column - Restex deactivated uncoated fused silica gel, 5 m, 0.53 mm ID, Catalog #10045
-Column Connection - Supelco Glass Seal Connector, Catalog #2-0479
-Gas Flows - Nitrogen (makeup) 20 mL/min, Nitrogen (column) 3.5 mL/min
-Temperatures - Injector 250 C, Detection 270 C, Column 215 C, Initial Hold 1.0 min
-Column Program - Final temp 250 C, Rate 5.0 C/min, Hold 25.0 min, Total time 33 min
-Electron Capture Detection - Attenuation 32, Range 10
-Retention Times - RH-0671 19.2 min; RH-2382 19.8 min; RH-2915 21.8 min; RH-4672 22.0 min

The registrant did not state which column was used for analysis and which was used for confirmation. The comments noted in the review of the meat method are applicable here too (Rtx-2300 = 90% biscyanopropyl/10% phenylcyanopropyl polysiloxane). Unless a confirmatory method that differs more from the analytical method is developed (preferably GC/MS with selected ion monitoring), interference testing will be required.

Preparation of Standard Curves - Instructions are as follows:

"Standard solutions of RH-2915/RH-0671/RH-2382/RH-4672in toluene are prepared (by serial dilution) in the concentration range 0.010 ug/mL - 0.20 ug/mL. Prepare a minimum of 5 standards within the concentration range. One or two microliters of each standard solution are injected and the resulting standard curve is constructed by plotting peak heights measured versus concentration (ug/mL). The

standard curves are constructed by quadratic regression within the concentration range. Standard curves are prepared for each analysis day."

CBRS concludes that the registrant must rewrite these instructions with more detail, as was the case for the meat method above and the egg method reviewed below.

Also as for the meat method, CBRS concludes that the registrant should use linear regression analysis for construction of standard curves.

Quantitation One or two microliters of the sample consistent with the volume injection for the standards are injected into the GLC. If necessary, the sample is diluted to an appropriate volume to give a response within the standard curve range. The peak height is measured and the concentration is determined from the standard curve. The registrant stated that the concentration is then determined as follows:

$$\text{Eq. 1} \quad \frac{\text{Total volume (mL)} \times \text{Concentration (ug/mL)} \times 100}{\text{Average Recovery (\%)} \times \text{Sample weight (g)}} = \text{ppm}$$

CBRS notes that Branch policy does not allow for correcting for percent recovery. Equation 1 must be modified to remove average percent recovery from the denominator. Additionally, for clarity and consistency (see Eq. 2 below) the registrant should change "Total volume (mL)" to "Final sample volume (mL)".

Fortification The registrant states,

"For samples fortified with known amounts of RH-2915/RH-0671/ RH2382/RH-4072 prior to extraction, measure peak height, determine the ug/mL from the standard curve, correct for any background in the control sample, and calculate percent recovery from equation 2.

$$\text{Eq. 2} \quad \frac{[\text{(ug/mL) found} \times \text{Final sample volume (mL)} - \text{ug control}] \times 100}{\text{ug added}} = \text{\% Recovery"}$$

CBRS prefers that when analyses are corrected for matrix effects (background corrections) both corrected and uncorrected results be reported (see below).

Results

Representative standard chromatograms, standard curves, control milk chromatograms, fortified milk chromatograms and treated milk sample chromatograms (samples from metabolism study) were provided for both GLC columns.

Standard Curves - Although representative standard curves were provided for each analyte/column combination, the raw data (peak heights and ug/mL) used to construct the curves were not provided. This is a deficiency. All raw data used to construct a

representative standard curve should be provided (one set of data for each GLC column). As noted above, CBRS prefers that linear regression analysis be used instead of quadratic regression.

Fortifications - Recovery data for fortifications are summarized in Table 4. The registrant proposed 0.01 ppm as the tentative limit of quantitation (LOQ). Based on the available data, an LOQ of 0.01 ppm appears to be appropriate for residues of oxyfluorfen in milk. For residues of the isomers RH-2382, RH4672, and RH-0671 in milk, 0.02 ppm appears to be an appropriate LOQ. CBRS reserves final judgement on this issue until receipt of sample identification information requested below.

CBRS notes that the registrant did not specify which GLC column was used to generate the data presented in Table 1. This is a deficiency. Additionally, both uncorrected and background corrected results must be presented. CBRS also notes that from the way the data are presented it is unclear if three separate samples were analyzed at the indicated fortification levels or if the same sample was analyzed three times.

The registrant should provide a more detailed data summary table, including sample identification codes, dates of extractions, dates of analysis, and any other pertinent information. The registrant did not supply an example calculation for one of the fortified samples. An example calculation, including all supporting data (chromatograms, peak height measurements, standard curve used, etc.) must be provided.

Table 4. Summary of Fortification Data.

Fortification Level (ppm)	Percent Recovery			
	Oxyfluorfen	RH-0671	RH-2382	RH-4672
0.010	114	140	82	68
	114	98	104	90
	70	60	55	61
Average	99	99	80	73
0.020	126	120	103	98
	89	82	93	98
	105	102	100	102
Average	107	101	99	99
0.050	117	105	106	92
	104	109	106	109
	82	86	85	82
Average	101	100	99	94

Fortification Level (ppm)	Percent Recovery			
	Oxyfluorfen	RH-0671	RH-2382	RH-4672
0.010	93 86 109	83 89 112	82 87 107	87 87 111
Average	96	95	92	95
0.050	89 72 98	86 75 95	85 73 100	85 75 --
Average	86	85	86	80

Radiovalidation - Table 5 presents results of the method radiovalidation using samples obtained from the metabolism study. The registrant did not identify which samples from the metabolism study were analyzed. This is a deficiency.

In the metabolism study (MRID #42670601), results for day 4 milk were reported to be 0.209 ppm and 0.142 ppm respectively for chlorophenyl and nitrophenyl ^{14}C-labeled oxyfluorfen. Oxyfluorfen was extracted from milk using chloroform/methanol (1:2, v/v). The CH_3Cl/MeOH fraction was concentrated, dissolved in heptane, and partitioned with acetonitrile. Oxyfluorfen was found in the ACN fraction.

The chlorophenyl and nitrophenyl ^{14}C-labeled oxyfluorfen used in the metabolism study had radiochemical purities of 100% and 95% respectively, that is, none of the isomers were present to a significant extent. Until the registrant can provide additional data supporting the proposed LOD of 0.003 ppm for the analytical method, the non-detectable results (<0.003 ppm) listed in Table should be changed to <0.02 ppm.

Table 5. Radiovalidation results for milk analytical method.

Analysis	ppm			
	Oxyfluorfen	RH-0671	RH-2382	RH-4672
Metabolism	0.19	<0.003	<0.003	<0.003
Residue	0.13	<0.003	<0.003	<0.003

Table 6 presents results for extraction efficiency of the analytical method. Again CBRS notes that the samples from the metabolism study were not identified. This is a deficiency. It is not clear if the samples labeled A and B are the same samples or different samples. The registrant must provide sample identification information, as well as pertinent dates (for example, date of extraction and storage time/condition of samples prior to extraction).

Table 6. Extraction efficiency.

Sample	dpm			% Extraction Efficiency
	Hexane	Aqueous	Total	
Milk-^{14}C-A	33,100	1400	34,500	96
Milk-^{14}C-B	28,900	3000	31,900	91

Egg Analytical Method (MRID #43307503)

Reference Standards - No information concerning the purity of the reference standards or lot numbers was provided. This is a deficiency. Additionally, information concerning the standards must be supplied as noted above for the meat study.

Extraction - Whole eggs (egg yolk and white) are prepared by blending at low speed with a Waring Blender for 1 to 2 minutes. The samples are stored frozen until used. Before analysis, samples are thawed and homogenized for about 1 minute to obtain homogeneous samples. The registrant described the extraction procedure as follows:

> "Weigh 5.0 g of sample into a 250 ml glass bottle. Add 100 mL of acetonitrile and homogenize with a Tissumizer at medium speed for 2 min. Filter the samples under suction with a vacuum pump (about 50 mm Hg) through Whatman #41 filter paper which has been prewashed with ACN and dried. Wash the bottle twice with 25 mL ACN and filter.
>
> The filtrate is transferred to a 500 mL separatory funnel containing 100 mL of petroleum ether. Shake vigorously for 2 minutes. This step is critical, because not shaking enough will result in low recovery. An alternative procedure to the shaking is to homogenize the mixture with a Tissumizer for 2 min before filtering. Add 10 mL saturated NaCl and 200 mL of distilled water to the mixture and shake gently (holding the funnel in a horizontal position) for about 45 sec. If three phases occur, add an additional 50 mL of water and mix it again. After the phases are separated, transfer the aqueous phase to another separatory funnel and extract twice with 50 mL of petroleum ether. Combine all petroleum ether extracts in a separatory funnel. Gently wash the petroleum ether extracts twice with 100 mL of distilled water. Finally, collect the petroleum ether extract in a 500 mL round bottom flask and concentrate to about 10 mL using a rotary evaporator (50 C water bath).

Florisil Column Chromatography - Instructions are as follows:

> "Prepare an activated florisil column (10 cm with an additional 2 cm of Na_2SO_4 on the top, column dimensions: 30 x 1.9 cm ID) be adding the florisil the Na_2SO_4 into a glass column containing 40 mL of petroleum ether. Pass the concentrated petroleum ether extract through the column at a rate of about 5 mL/min (about the same rate is applied for the subsequent washes and elution). When the extract reaches the top of the column, wash the round bottom flask twice with 25 mL of petroleum ether and pass through the column. The column is then washed with 50 mL of 15% diethyl ether in petroleum ether. Discard all washes up to this point. The GOAL residues are eluted from the column by 50 mL of 50% diethyl ether in petroleum ether. The eluent is evaporated to about 1 mL using a rotary evaporator (50 C water bath). Add about 20 mL of hexane to the round bottom flask and continue to evaporate to about 1 mL. Bring up to a final volume of 5 mL with hexane for GC-ECD analysis."

GLC Chromatography - A Hewelett Packard 5890 Series II GC equipped with a Model 7673 Autosampler and an Electron Capture Detector was used. Data were obtained with a HP 300 Data Acquisition and Processing Station with HP Extrachrom Software. Data were processed with Nelson Analytical Software.

Two different columns are used:

Column 1:

-Analytical Column - Restex Rtx-200, 0.32 mm ID, 60 m, 1.0 u film, Catalog #15057
-Gas Flows - Nitrogen (makeup) 40 mL/min, Helium (column) 3.5 mL/min
-Temperatures - Injector 265 C, Detection 300 C, Column 150 C, Initial Hold 1.0 min
-Column Program - Final temp 250 C, Rate 10.0 C/min, Hold 16.0 min
-Electron Capture Detection
-Retention Times - RH-0671 21.4 min; RH-2382 22.1 min; RH-2915 23.5 min; RH-4672 23.9 min
-Injection Volume - 1uL

Column 2:

-Analytical Column - Restex Rtx-50 megabore Column, 0.32 mm ID, 60 m, 1.0 u film, Catalog #10557
-Gas Flows - Nitrogen (makeup) 40 mL/min, Helium (column) 3.5 mL/min
-Temperatures - Injector 265 C, Detection 300 C, Column 215 C, Initial Hold 1.0 min
-Column Program - Final temp 250 C, Rate 5.0 C/min, Hold 25.0 min
-Electron Capture Detection
-Retention Times - RH-0671 22.7 min; RH-2382 21.4 min; RH-2915 23.8 min; RH-4672 24.5 min
-Injection Volume - 1 uL

Concerning the confirmatory method, the comments noted in the review of the meat method are applicable here too. Unless a confirmatory method that differs more from the analytical method is developed (preferably GC/MS with selected ion monitoring), interference testing will be required.

Preparation of Standards - Instructions are as follows:

"Prepare standard solutions by weighing on an analytical balance 10 mg each of analytical standards RH-2915, RH-0671, RH-2382, and RH-4672 (corrected for percent purity of each compound) into individual 10 mL volumetric flasks. Dissolve each of the compounds in about 5 mL of methanol and bring to the final volume with methanol. The resulting solutions are 1mg/mL stock solutions.

Transfer 0.1 mL of each of the stock solutions into a 10 mL volumetric flask and bring to volume with methanol. This results in an intermediate standard containing 10 ug/mL of each of RH-2915, RH-0671, RH-2382, and RH-4672.

Prepare working standard solutions of 0.005, 0.01, 0.02, 0.05 and 0.10 ug/mL by transferring 5, 10, 20, 50, and 100 uL of the intermediate standard into a 10 mL volumetric flask and bring to volume with hexane. Standards can also be made by serial dilution.

To prepare standard curves, 1 uL of each standard solution is injected and the resulting standard curve is constructed by plotting peak heights measured versus concentration (ug/mL). The standard curves are constructed by quadratic regression within the concentration range. Standard curves are prepared for each analysis day.

As for the meat method, CBRS concludes that linear regression analysis should be used for construction of standard curves. The registrant should modify the method write-up appropriately.

Quantitation One microliter of the sample consistent with the volume injection for the standards are injected into the GLC. If necessary, the sample is diluted to an appropriate volume to give a response within the standard curve range. The peak height is measured and the concentration is determined from the standard curve. The registrant stated that the concentration is then determined as follows:

$$\text{Eq. 2} \quad \frac{\text{Total volume (mL)} \times \text{Concentration (ug/mL)} \times 100}{\text{Average Recovery (\%)} \times \text{Sample weight (g)}} = \text{ppm}$$

CBRS notes that Branch policy does not allow for correcting residue results for percent recovery of concurrent fortifications. Equation 2 must be modified to remove average percent recovery from the denominator. Additionally, for clarity the registrant should change "Total volume (mL)" to "Final sample volume (mL)".

Fortification To determine percent recovery, Equation 3 is used:

$$\text{Eq. 3} \quad \frac{[\text{Concentration (ug/mL)} \times \text{Total volume (mL)} - \text{ug in control}] \times 100}{\text{ug added}} = \text{\% Recovery"}$$

CBRS prefers that when analyses are corrected for matrix effects (background corrections) both corrected and uncorrected results be reported.

Results

Representative standard chromatograms, standard curves, control egg chromatograms, and fortified egg chromatograms were provided for both GLC columns.

Standard Curves - Although representative standard curves were provided for each analyte/column combination, the raw data (peak heights and ug/mL) used to construct the curves were not provided. This is a deficiency. All raw data used to construct a representative standard curve should be provided (one set of data for each GLC column). As noted above, CBRS prefers that linear regression analysis be used instead of quadratic regression.

Fortifications - Recovery data for fortifications are summarized in Tables 7 and 8. The registrant proposed 0.01 ppm as the tentative limit of quantitation (LOQ), but did not provide a statistical justification or sufficient data to support this proposal.

Both uncorrected and background corrected results must be presented.

The registrant should provide a more detailed data summary table, including sample identification codes, dates of extractions, dates of analysis, and any other pertinent information. The registrant did not supply an example calculation for one of the fortified samples. An example calculation, including all supporting data (chromatograms, peak height measurements, standard curve used, etc.) must be provided.

CBRS notes that for all analyte fortifications $\geq$ 0.010 ppm, with two exceptions (noted in shading in the summary tables) recoveries are generally in the range considered acceptable by the Agency (70 - 120%).

Table 7. Summary of Fortification Data obtained using the Rtx-200 Analytical GC Column.

Fortification Level (ppm)	Percent Recovery			
	Oxyfluorfen	RH-0671	RH-2382	RH-4672
0.010	90.6, 86.3 75.5, 70.2 84.3, 78.0 86.7, 91.8 84.4, 84.7 78.0, 76.0	85.7, 72.3 77.2, 72.3 83.9, 83.6 90.4, 97.4 80.1, 78.8 82.8, 77.4	95.0, 89.9 84.4, 78.6 86.1, 67.1 87.4, 96.4 82.4, 83.6 78.1, 80.6	85.5, 81.1 76.7, 70.6 79.7, 76.6 86.0, 89.4 79.9, 79.9 73.7, 73.7
0.050	85.2, 78.6 90.8, 91.4 71.8, 80.8 102, 96.6 81.8, 84.8 95.6, 92.0	83.2, 76.2 92.4, 90.2 76.0, 83.8 100, 97.8 80.7, 88.1 96.2, 88.4	86.6, 78.2 83.0, 86.4 48.6, 81.0 101, 93.1 86.0, 84.6 91.6, 88.0	82.0, 77.4 88.2, 88.2 71.4, 78.8 96.6, 94.0 63.8, 82.2 91.4, 86.2
0.100	93.6, 88.9 84.2, 84.5 81.3, 84.3	95.6, 92.1 86.1, 85.4 74.4, 84.2	94.1, 88.6 84.3, 83.0 79.8, 86.3	91.8, 87.0 82.3, 83.0 71.0, 82.3
1.00	83.4, 84.2 77.8, 91.0 99.2, 103 95.0, 82.2 85.6, 91.2 93.0	79.2, 81.0 75.6, 88.8 99.6, 104 99.6, 81.5 88.5, 85.4 90.4	82.8, 73.4 76.2, 86.0 96.8, 100 93.2, 80.1 84.9, 86.8 88.4	81.8, 80.8 75.6, 87.6 96.2, 103 96.6, 80.0 84.2, 87.2 91.8
5.00	95.0, 89.4 86.8, 94.6 91.2, 94.8	96.8, 92.6 88.0, 94.8 88.4, 89.0	92.2, 87.4 86.4, 90.4 87.2, 89.2	96.8, 92.6 88.0, 94.8 88.4, 89.0

Table 8. Summary of Fortification Data obtained using the Rtx-50 Analytical GC Column.

Fortification Level (ppm)	Percent Recovery			
	Oxyfluorfen	RH-0671	RH-2382	RH-4672
0.010	74.7, 74.7 80.6, 76.3 96.3, 101	100, 96.2 81.9, 68.5 109, 115	69.2, 67.7 73.7, 65.5 73.5, 80.5	60.6, 65.1 37.7, 81.9 87.5, 86.6
0.050	76.2, 77.2 89.6, 79.2 98.6, 93.8	76.8, 87.8 87.2, 78.4 96.5, 97.3	80.4, 78.6 88.2, 77.4 96.4, 90.0	58.6, 73.2 85.8, 77.0 92.0, 87.4
0.100	71.1, 68.8 86.6, 82.5	74.9, 76.7 88.2, 87.9	71.7, 68.2 85.6, 83.0	68.3, 66.2 85.2, 80.9
1.00	94.0, 99.6 91.4, 77.4 92.0, 91.2	80.2, 87.8 87.2, 71.6 92.3, 92.3	78.6, 85.8 90.4, 75.4 94.0, 91.0	81.0, 87.8 90.8, 75.0 90.8, 88.6
5.00	116, 116 92.8, 92.0	99.2, 104 91.1, 90.5	101, 101 92.2, 91.8	105, 108 91.8, 88.0

Radiovalidation - The method was not radiovalidated using egg samples from the metabolism study. Eggs from the poultry metabolism study contained 1.037 and 1.026 ppm ^{14}C-oxyfluorfen (chlorophenyl and nitrophenyl ring labeled respectively).

Response to Review of Ruminant Metabolism Study

The review of the goat metabolism study (S. Knizner, 6/16/93, CBRS #11526, MRID #42670601) noted the deficiencies listed below. These deficiencies are repeated (numbering system used in review), and the registrant's and CBRS response follow.

1b. CPR and NPR radioactive residues in milk were highest in the Day 4 sample, at 0.252 and 0.211 ppm, respectively. For the other 6 milk samples (days 1-3 and 5-7), average TRR levels in the CPR and NPR dosed goats were 0.059 ± 0.024 ppm and 0.063 ± 0.018 ppm respectively. The TRR level in the Day 4 milk sample is more than 6 standard deviations larger than the average results obtained on all other study days. In a preliminary review of results from the goat metabolism study (C.Olinger, 6/15/92, CBRS #9913), the registrant was asked to provide an explanation for the sudden increase in radioactive residues in the Day 4 milk. The registrant did not provide an explanation. This is a deficiency.

Registrant's Response: The registrant stated that the apparent spike in the Day 4 milk sample occurred for unknown reasons. They postulated that it may be due to a pharmacokinetic effect due to the absorption and elimination of oxyfluorfen because it was

observed in milk from both dosed goats, and oxyfluorfen was the major component of the spike. The fact that the levels seem constant for all other sample points analyzed demonstrates that this spike is transient. The registrant noted that the Day 2 CPR sample contained 67% of TRR as oxyfluorfen and the Day 6 sample contained 74.5% oxyfluorfen.

CBRS Response: The registrants explanation adequately addresses this deficiency. CBRS notes that in the dairy cattle feeding study (MRID #43152201, see S.Knizner, 8/19/94, CBRS #13395), where cows were dosed at lower levels (4x, 13x, and 43x versus the 168x used in the metabolism study), no "spikes" in oxyfluorfen concentrations were noted in milk over the 28 days of dosing.

2b. In liver, 1-6% of the TRRs from various samples were tentatively identified as oxyfluorfen. The registrant stated that further characterization and identification of liver metabolites is continuing. Adequacy of this study is reserved pending the submission of the additional liver data.

Registrant's Response: The registrant has submitted a supplemental study (MRID #43317701) for characterization of residues in liver. This study is reviewed below.

CBRS Response: The submitted study adequately characterized radioactive residues in liver. This deficiency is resolved.

3. Samples from the metabolism study were stored for up to 21 months prior to analysis. Storage stability data on milk and tissues are required to support the storage conditions and intervals of this study. Additionally, the registrant stated that a freezer thaw occurred for 48 hours where the temperature reached approximately 20 °C. Several liver subsamples and kidney samples were in the freezer. The registrant stated that documentation of the freezer thaw was included in the raw data. However, the reviewer could not find the necessary information. The registrant must identify the samples involved in the freezer thaw and demonstrate that this event did not influence the results of the study.

Registrant's Response: Storage stability studies for oxyfluorfen in milk, meat, and liver are in progress. These studies were initiated following analytical method development for the particular matrix. The milk study was initiated in July of 1993, the muscle study in December, 1993, and the liver study in February, 1994. All studies are two year studies.

Additionally, the registrant stated that for milk, the metabolite pattern for samples analyzed after 10 months of storage is essentially the same as for samples analyzed at 19-23 months. Supporting data for this statement exists, but was not provided.

Concerning the freezer malfunction, the registrant provided a table listing exactly which samples were affected. Only two NPR and two CPR liver subsamples and both kidney samples. The registrant stated that for both liver and kidney, samples were analyzed before

and after the thaw, and comparisons of the extractable residues suggest that the thaw had no effect.

CBRS Response: Until the required storage stability studies are submitted, reviewed, and found to be adequate, the deficiency concerning storage stability remains. CBRS concerns over the freezer thaw have been adequately addressed.

4. Representative samples from the goat metabolism study must be analyzed using enforcement analytical methods for radiovalidation purposes.

Registrant's Response: The registrant complied this with this requirement in their submitted meat/milk analytical enforcement method (MRID #43307502 and 43346401).

CBRS Response: The registrant has adequately resolved this deficiency (see review of meat/milk analytical enforcement method (MRID #43307502 and 43346401).

Addendum to Ruminant Metabolism Study, Supplemental Analysis of Liver Samples (MRID #43317701)

The review of the submitted ruminant metabolism study, MRID #42670601, (S.Knizner, 6/16/93, CBRS #11526) required further analysis of liver. In the current study, liver from the goat dosed with C^{14}-nitrophenyl ring labeled oxyfluorfen (NPR) was analyzed. The performing laboratory was XenoBiotic Laboratories, Inc. (XBL).

The goat liver sample was shipped from ABC Laboratories (the performing lab for the in-life phase) to Rohm and Haas on 10/15/91. Samples were stored frozen at -20 C. Samples were shipped to XBL on 3/24/93 and stored frozen until analysis.

Reference Standards The reference standards used in this study include oxyfluorfen, chlorophenol (RH-34800), amino-hydroxy-oxyfluorfen (RH-45298), hydroxy-oxyfluorfen (RH-670), 4'-acetamido-oxyfluorfen (RH-35450), acetamido-oxyfluorfen (RH-45469), and amino-oxyfluorfen (RH-35451). The purity and lot number of each standard was provided. The structures of the standards are presented below in Figure 1.

Figure 1. Structures of standards used for metabolite identification.

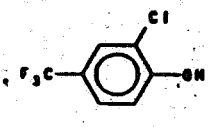
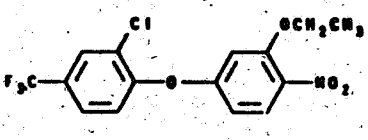
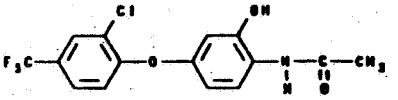
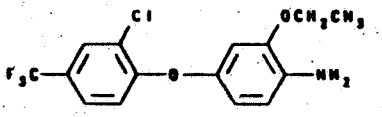
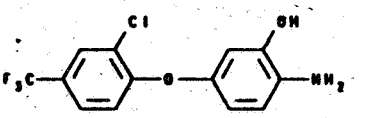
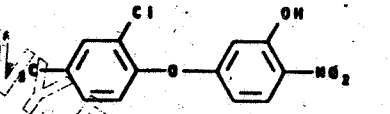
Chemical Name	Sponsor Number	Purity (%)	Lot No.	Chemical Structure
3-Chloro-4-hydroxy-benzotrifluoride (Chlorophenol)	RH-34800	93.8	DJH042	
GOAL® (Oxyfluorfen)	RH-32915	99.4	RP98674FP	

Figure 1 (cont.). Structures of standards used for metabolite identification.

Chemical Name	Sponsor Number	Purity (%)	Lot No.	Chemical Structure
Acetamido RH-670 (Acetamido-670) or (N-Acetyl-670) or (N-Acetyl-hydroxy-oxyfluorfen) or (N-Acetyl-hydroxy-GOAL)	RH-45469	91.8	HGW74:77B	
Amino-GOAL	RH-35451	98.2	MD7:46C	
Amino-hydroxy-GOAL (Amino-670) or (Amino-hydroxy-oxyfluorfen)	RH-45298	97.5	HGW74:46B	
RH-670, GOAL Intermediate (OH-oxyfluorfen) or (OH-GOAL)	RH-34670	99.9	RH-6660-1	
4'-Acetamido GOAL (N-acetyl GOAL) or (N-acetyl-oxyfluorfen)	RH-35450	99.6	MD7:42D	

Analytical

Combustion Analysis - Harvey Model OX-300 and)X-500 biological Sample Oxidizers were used for combustion analyses.

HPLC - A Waters Model 484 HPLC, using a Ultracarb ODS-20 column both a UV and a Raytest Raymona-5 Radioactivity Monitor was used for HPLC analyses. Two different solvent gradient programs were used to elute compounds of interest. An ISCO Foxy Fraction Collector collected fractions every 30 seconds, and following LSC counting, reconstructed HPLC chromatograms were made. A table was provided listing the R_fs of all reference compounds.

TLC - 2D-TLC was used for qualitative confirmation and characterization of metabolites isolated using HPLC. Normal phase silica gel plates were used. Collected fractions and reference standards were spotted on the plates and then developed in two dimensions. Four different solvent systems were used. A table was provided listing the R_fs of all reference standards.

Extraction and Fractionation

Liver was blended with MeOH/H_2O/$CHCl_3$ (11:5:5). The tissue was first blended with MeOH/H_2O, then $CHCl_3$ was added and blended again. The mixture was separated by filtration. The solids were reblended with $CHCl_3$ and filtered. The filtrates were combined and the layers separated using a separatory funnel to yield a $CHCl_3$ and MeOH/H_2O fraction. The $CHCl_3$ fraction was evaporated to near dryness, followed by addition of hexane/ACN, to yield a hexane and ACN soluble fraction.

The PES were subjected to protease digestion (Protease Type I, Sigma), followed by centrifugation. The supernatant obtained (AQ-3) was cleaned up by solid phase chromatography with Amberlite XAD resin. The centrifugation pellet (PES-2) was subjected to hydrolysis with 1 N HCl (refluxed under nitrogen for 4 hours). The sample was then centrifuged to yield and aqueous soluble fraction AQ-5 and PES-3. The PES-3 fraction was then hydrolyzed with 6 N HCl using the same conditions as for the 1 N HCl digestion (except digestion was carried out for 24 hours). The resulting digest was centrifuged to yield AQ-6 and PES-4 fractions.

The hexane fraction of the $CHCl_3$ extract was subjected to saponification using 1 M KOH in 95% ethanol. The mixture was refluxed for 2 hours. Upon cooling, water was added and the mixture was then extracted with diethyl ether to yield a Diethyl Ether-1 (nonsaponifiable) and AQ-1 (saponifiable) fraction. The AQ-1 fraction was acidified then extracted with diethyl ether to yield a Diethyl Ether-2 and an AQ-2 fraction.

Results

The TRR obtained for liver in this study was 0.406 ppm. This is in good agreement with the level reported in the original metabolism study (0.378 ppm).

Table 9 summarizes the distribution of radioactive residues in the various fractions and their characterization/identification. Table 10 presents total amounts of the various metabolites identified and characterized.

CBRS concludes that the registrant has adequately characterized/identified radioactive residues in ruminant liver. The predominant liver metabolites identified were amino-oxyfluorfen (and its conjugates) and amino-hydroxy-oxyfluorfen (and its conjugates).

Table 9. Distribution and characterization/identification of radioactive residues in the various fractions. TRR was 0.406 ppm.

Fraction	% TRR	ppm	Characterization/Identification of Residues
MeOH/H_2O	22.0	0.089	HPLC analysis showed 7 peaks at 0.004 - 0.031 ppm (only 2 peaks were >0.010 ppm). 2-D TLC analysis indicated the presence of 6 metabolites, RH-45298 conjugate (3.0% TRR, 0.013 ppm), RH-35451 conjugate (4.9% TRR, 0.020 ppm), diconjugate of RH-45298 (7.7% TRR, 0.031 ppm), and 3 unknowns (0.004 - 0.007 ppm)
$CHCl_3$	36.5	0.148	
ACN	16.7	0.069	RH-35450 at 4.3% TRR (0.018 ppm), and 4 unknowns (at 0.9% TRR, 0.004 ppm, to 5.7% TRR, 0.023 ppm).
Hexane	15.0	0.061	
Diethyl Ether-1	6.9	0.028	RH-35450 0.5% TRR 0.002 ppm, RH-45298 0.6% TRR, 0.002 ppm, 8 unknowns,ranging from 0.002-0.005 ppm
AQ-1	11.9	0.048	
Diethyl Ether-2	8.1	0.032	Not Analyzed
AQ-2	2.9	0.012	Not Analyzed
PES-1	50.4	0.205	
AQ-3	10.2	0.041	
MeOH-1	3.7	0.015	Conjugate of RH-35451 (0.5% TRR, 0.002 ppm), diconjugate of RH-45298 (1.1% TRR, 0.004 ppm) and 3 unknowns (all at 0.002 ppm).
AQ-4	7.4	0.030	Not Analyzed
PES-2	40.0	0.162	
AQ-5	14.2	0.058	RH-45298 (1.2% TRR, 0.005 ppm), RH-35451 (2.6% TRR, 0.011 ppm) RH-45298 conjugate (1.5% TRR, 0.006 ppm), RH-35451 conjugate (1.7% TRR, 0.012 ppm), diconjugate of RH-45298 (3.0% TRR, 0.012 ppm), and 3 unknowns (0.003 - 0.004 ppm)
PES-3	24.5	0.099	
AQ-6	13.3	0.054	RH-352450 (1.0% TRR, 0.004 ppm), RH-35451 (4.1% TRR, 0.017 ppm) RH-45298 conjugate (1.2% TRR, 0.005 ppm), RH-35451 conjugate (2.0% TRR, 0.008 ppm), diconjugate of RH-45298 (2.0% TRR, 0.008 ppm), and 3 unknowns (0.004 - 0.006 ppm)
PES-4	8.0	0.032	Not Analyzed
Total	**108.9**	**0.442**	

Table 10. Amounts of various metabolites characterized/identified in ruminant liver.

Metabolite	% TRR	ppm
RH-35450	5.9	0.024
RH-45298 or RH-45469	1.7	0.007
RH-35451	6.7	0.028
RH-35451-conjugates	10.2	0.041
RH-45298-conjugates	19.4	0.078
Non-polar unknowns[a]	11.1	0.044
Organosoluble Unknowns[b]	4.5	0.018
Aqueous-Soluble Unknowns[c]	15.0	0.061
Not Analyzed or Lost During Fractionation	25.6	0.105

[a] Three unknown metabolites found in the ACN fraction (0.9% - 5.7% TRR, 0.003 - 0.023 ppm).
[b] Metabolites found in Diethyl Ether-1 fraction (8 unknowns ranging from 0.002 - 0.005 ppm).
[c] Six metabolites ranging from 0.8 - 3.9% TRR (0.003 - 0.16 ppm).

cc: S.F., circ., R.F., List B File, S.Knizner
RDI: W. Smith 10/31/94, B. Cropp-Kohlligian 10/31/94, L.Edwards 10/31/94, P.Deschamp 10/31/94, C.Olinger 10/31/94, M.Metzger, 11/8/94 E.Zager, 11/11/94
7509C:CBRS:CM#2:305-6903:SAK:sak:Oxyfluor:10/31/94