CONFIDENTIAL 1-26-94

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

JAN 26 1994

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM:

SUBJECT: PP#3G4224/000241-EUP-REA - New Chemical - Pirate Insecticide on Cotton. Evaluation Of Product and Residue Chemistry Data. MRID Nos. 427702-01 thru 06 and 427702-34 thru thru 39. CBTS Nos. 12023, 12292, and 12293. DP Barcodes D192275, D193604 and D193607.

FROM: Gary F. Otakie, P.E., Chemist
Tolerance Petition Section II
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Gary F. Otakie

THRU: Debra F. Edwards, Ph.D., Chief
Chemistry Branch I - Tolerance Support
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Robert Schmitt for

TO: Dennis Edwards/Meredith Johnson, PM 19
Insecticide - Rodenticide Branch
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and

Albin Kocialski, Head
Registration Section
Chemical Coordination Branch
Health Effects Division (H7509C)

Attached are the Residue and Product Chemistry reviews for the new chemical Pirate an insecticide for use on cotton prepared by Dynamac Inc. and reviewed by CBTS. This review has undergone secondary review and revision in the Chemistry Branch and reflects Agency policies.

Although CBTS has no objection to granting the requested EUP and the establishment of the proposed temporary tolerance of 0.5 ppm for the parent Pirate in/on cottonseed for a period of two years, several deficiencies discussed in the attached reviews will require resolution for approval of any permanent tolerance on Pirate.

If you need additional input please advise.

Attachment 1: Residue Chemistry Data Submitted in Support of an Experimental Use Permit and Temporary Tolerance for Cottonseed; 10/22/93.

Attachment 2: Proposed Metabolic Pathway for Pirate in Ruminants and Poultry.

Attachment 3: International Residue Limit Status Sheet

Attachment 4: Product Chemistry Data Submitted in Support of an Experimental Use Permit and Temporary Tolerance for Cottonseed; 10/22/93.

Attachment 5: Confidential Appendix - Pirate Product Chemistry

cc with Attachments 1, 2, 3, and 4: R.Griffin, Circu.

cc with Attachment 1, 2, 3, 4, and 5: Reviewer-Otakie, PP#3G4224, RF, E. Haeberer, P. Deschamp.
RDI: EHaeberer:1/13/94 RLoranger:1/21/94

ATTACHMENT 1

DYNAMAC CORPORATION
Environmental Services

Final Report

PIRATE (CBTS)

TASK 4
Residue Chemistry Data Submitted in Support of an Experimental Use Permit and Temporary Tolerance for Cottonseed

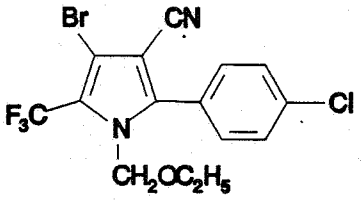
October 22, 1993

Contract No. 68-D2-0053

Submitted to:
U.S. Environmental Protection Agency
Arlington, VA 22202

Submitted by:
Dynamac Corporation
The Dynamac Building
2275 Research Boulevard
Rockville, MD 20850-3268

PIRATE



Task 4

REQUEST FOR AN EXPERIMENTAL USE PERMIT AND TEMPORARY TOLERANCE FOR COTTONSEED

INTRODUCTION

American Cyanamid Company has petitioned for an experimental use permit (EUP) and a temporary tolerance for residues of the insecticide and miticide Pirate™ [4-bromo-2-(chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile] as follows:

Cottonseed . . . . . 0.5 ppm

No temporary or permanent tolerance has yet been established. The proposed EUP permits the use of up to 5,226 lb ai of Pirate (1,742 gallons of formulated product) on 3,990 acres of cotton in the southeastern, south central, and southwestern U.S. per year over a two year period beginning in 1994.

CONCLUSIONS

1. For purposes of this temporary tolerance petition, the nature of the residue in plants is adequately understood. The residue to be regulated is the parent compound Pirate.

   For establishment of a permanent tolerance, the nature of the residue is not adequately understood. Raw data are needed showing the amount of radioactivity in individual unknown peaks detected in organosoluble extracts of cottonseed meal and linters. In addition, data must be submitted demonstrating the stability of ^{14}C-residues in samples stored for up to 545 days (~18 months). The petitioner indicated that the storage intervals for cottonseeds prior to analysis ranged from 40 to 501 days. HPLC chromatograms showing that 40- and 501-day metabolite profiles are similar would fulfill this requirement. In addition, the proposed enforcement method must be radiovalidated using representative samples from the cotton metabolism study. The HED Metabolism Committee will also be consulted as to which residues should be regulated in plant and animal commodities.

2a. For purposes of this EUP and temporary tolerance petition only, CBTS concludes that tolerances on animal commodities are not required since residues of Pirate from the subject EUP are not likely to occur in animal commodities and therefore the qualitative nature of the residue in ruminants is not an issue for the subject EUP (see Conclusion No. 7).

For establishment of a permanent tolerance, the nature of the residue in goats is not adequately understood. Radioactive residues released by acid and base hydrolyses from liver (10.3-40% TRR; 0.15-0.58 ppm) and kidneys (13.8-41.3% TRR; 0.14-0.26 ppm) must be further characterized. In addition, the petitioner must provide the storage conditions and the dates of extraction and analysis for samples from the goat metabolism study.

2b. For purposes of this EUP and temporary tolerance petition only, CBTS concludes that tolerances on poultry are not required since residues of Pirate from the subject EUP are not likely to occur in poultry and therefore the nature of the residue in poultry is not an issue for the subject EUP (see Conclusion No. 7).

For establishment of a permanent tolerance, the nature of the residue in laying hens is not adequately understood. Radioactive residues released from poultry liver by acid hydrolysis (14-47% TRR; 0.18-0.62 ppm) and from kidneys by enzymatic proteolysis (13-14% TRR; 0.05-0.07 ppm) require further characterization. In addition, the petitioner must provide the storage conditions and the dates of extraction and analysis for samples from the hen metabolism study.

2c. If tolerances on animal commodities are required for the issuance of a permanent tolerance on cottonseed, the proposed data collection and tolerance enforcement methods must be radiovalidated using representative samples from the goat and hen metabolism studies.

3a. The available data indicated that the GC/electron capture detection (ECD) methods M-2216 and M-2274 are adequate for determining residues of parent Pirate in/on cottonseed and in cottonseed processed commodities, respectively. The validated limit of detection for both methods is 0.05 ppm. These methods are adequate for enforcement of the tolerance expression (parent only) for this EUP and temporary tolerance petition. Radiolabeled validation of the proposed enforcement method is required for a permanent tolerance.

3b. A successful independent laboratory validation has been completed for the GC/ECD method M-2216; and the method has been forwarded for EPA Petition Method Validation. Prior to EPA laboratory validation, the petitioner must submit a reference standard of Pirate to the EPA repository, as well as the accompanying material safety data sheet (MSDS). Pirate should also be tested using FDA Multiresidue methods (PAM Vol. I).

4. For purposes of this EUP and temporary tolerance petition, the submitted storage stability data are adequate. The data indicate that residues of Pirate are stable under frozen storage conditions for up to 6 months. No storage stability data for residues of Pirate in cottonseed processed commodities were submitted. Cottonseed samples from the field residue and processing studies were stored frozen for 1.6-9.8 months prior to analysis, and processed commodities were analyzed after approximately 4 months of frozen storage. For establishment of a permanent tolerance, additional storage stability data are required reflecting the maximum storage interval for cottonseed (i.e. 10 months) and the stability of Pirate in processed matrices stored frozen for up to 4 months.

5. For purposes of this EUP and temporary tolerance petition, the available data indicate that residues of the parent Pirate are not likely to exceed the proposed temporary tolerance of 0.5 ppm in/on cottonseeds harvested 21 days following the last of 5 foliar broadcast applications of Pirate each at 0.4 lb ai/A, for a seasonal maximum rate of 2 lb ai/A. For a permanent tolerance additional field trial data representing commercial application procedures will be needed. A minimum of an additional 5 field trials in geographically representative areas are

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required with at least one field trial conducted in either Georgia, North Carolina or South Carolina. It is recommended that two composite samples be collected in each field trial.

6a. Although the processing study indicates that residues of the parent Pirate do not concentrate in cottonseed processed commodities several study questions need to be resolved. The submitted processing data are only marginally adequate for the subject EUP only. See body of review for detailed comments. Accordingly, no feed/food additive tolerances will be required for cottonseed processed commodities for the subject EUP only.

6b. An explanation of the discrepancy in the mass balance needs to be resolved (i.e. significantly higher Pirate residues in the RAC than in any of the processed fractions). A new mass balance providing the disposition of 90 to 100% of the Pirate in the cottonseed used for processing is required. If Pirate instability is claimed to be a major reason for the discrepancy in the mass balance, data on the stability of Pirate in hexane at solvent extraction temperatures and durations representative of commercial processing procedures and identification of resulting metabolites and or decomposition products will be needed. Additional information on the processing study are required such as residue levels in the delinted cottonseed used for processing as well as residues in the lint and trash, copies of the sponser processing protocol and the exact protocol used including all deviations made and the complete processing report (i.e. including the temperature and duration of solvent extraction and equipment used), original processing data and calculations as well as submission of data depicting the frozen storage stability of residues of Pirate in cottonseed processed commodities compared to sample storage intervals used in the processing study. A new cottonseed processing study may be required for a permanent tolerance unless these issues are adequately resolved.

7. For the purposes of this EUP and temporary tolerance petition only, CBTS concludes that residues of Pirate are not likely to be found in animal commodities, based on the feeding levels of the seven day metabolism studies and the resulting TRR. For the establishment of a permanent tolerance, a poultry feeding study is not likely to be required based on the total C14 residues in the metabolism study. However, a ruminant feeding study may be required (e.g. a common moiety method might result in detectable residues in the liver). A final determination of the secondary residues in meat, milk, poultry and eggs and the need for poultry or ruminant feeding studies is deferred until deficiencies relating to the nature of the residue in animals are resolved and a subsequent review by the HED Metabolism Committee is completed.

8. The proposed EUP label provides for cotton to be planted the season following Pirate applications and that the EUP studies should be established on continuous cotton rotation acreage. Accordingly, for the purposes of this EUP and temporary tolerance petition, CBTS concludes that crop rotation residue data are not required. However a confined crop rotation study and perhaps rotational crop field trials will be needed for a permanent tolerance.

9. For purposes of this EUP and temporary tolerance petition, the submitted product chemistry data are adequate. Product chemistry deficiencies requiring resolution for a permanent tolerance are discussed in detail in the attached Pirate Product Chemistry Review.

10. An International Residue Limit Status sheet is included in this review as Attachment 3. Since no Codex, Canadian, or Mexican limits/tolerances have been established for Pirate, there are no compatibility problems at this time.

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RECOMMENDATIONS

CBTS has no objection to granting the requested EUP and the establishment of the proposed temporary tolerance of 0.5 ppm for the parent Pirate in/on cottonseeds, for a period of two years.

For establishment of permanent tolerances, the petitioner must submit additional raw data and storage stability data from the cotton metabolism study (see Conclusion 1); submit further characterization of ^{14}C-residues and storage stability data from the goat and hen metabolism studies (see Conclusions 2a and 2b); validate the proposed enforcement method using representative samples from the cotton, goat, and hen metabolism studies (see Conclusions 1 and 2c); pass EPA Petition Method Validation (see Conclusion 3b); and submit storage stability data reflecting the maximum storage interval for cottonseeds and data depicting the stability of Pirate in processed matrices (see Conclusion 4); additional field trial data (see Conclusion 5); additional processing data (see Conclusion 6a. and 6b.); satisfy any feeding study requirements (see Conclusion 7); and submit additional product chemistry data (see Conclusion 9).

Prior to EPA laboratory validation of the proposed enforcement method, the petitioner must submit a reference standard of Pirate to the EPA repository, as well as the accompanying material safety data sheet (MSDS). Pirate should also be tested using FDA Multiresidue methods (PAM Vol. I).

DETAILED CONSIDERATIONS

Manufacture and Formulation

The manufacturing process and product chemistry date have undergone review by CBTS, and product chemistry data gaps have been identified which require resolution for a permanent tolerance. A complete discussion of the product chemistry of Pirate is included in the Pirate product chemistry review (see Attachments 4 and 5). Adequate product chemistry data are available for the subject EUP and temporary tolerance only.

Pirate Insecticide-Miticide contains 30.83% 4-bromo-2-(chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile and 69.17% inert ingredients. Pirate Insecticide-Miticide contains 3.0 lbs. of active ingredient (ai) per gallon. The molecular structure of Pirate is depicted on page 1.

Proposed Use

The 3 lb/gal EC formulation is proposed for multiple foliar applications to cotton at 0.07-0.4 lb ai/A. A maximum of 2 lb ai/A may be applied per year. Repeat application intervals of 5-7 days are listed for single application rates of 0.28-0.4 lb ai/A. Ground applications are to be made in a minimum of 5 gal/A and aerial applications are to be made in a minimum of 2 gal/A. A grazing restriction and a 21-day PHI are proposed. Cotton is the only crop that may be planted the season following Pirate applications

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Qualitative Nature of the Residue in Plants

Cotton

American Cyanamid (1993; MRID 42770234) submitted data depicting the metabolism of [2-pyrrole-^{14}C]-labeled and uniformly ring-labeled [phenyl-^{14}C]Pirate in cotton. The study was initiated in 1991 and conducted by Pan-Agricultural Laboratories, Inc. (PAL, Madera, CA). The radiolabeled test substances were diluted with non-radiolabeled Pirate containing [2-pyrrole-^{13}C]Pirate as a mass marker. The final specific activities for the [2-pyrrole-^{14}C]- and [phenyl-^{14}C]-test substances were 4.04 μCi/mg (8,969 dpm/μg; 98.1% radiochemical purity) and 4.02 μCi/mg (8,924 dpm/μg; 96.1% radiochemical purity), respectively.

Each test substance was formulated as a suspension concentrate and applied to field-grown cotton plants once a week for 5 consecutive weeks. The [2-pyrrole-^{14}C]-labeled test substance was applied at 0.37-0.43 lb ai/A/application, for a seasonal rate of 1.98 lb ai/A (~1x the maximum proposed seasonal rate). The [phenyl-^{14}C]-test substance was applied at 0.44-0.56 lb ai/A/application, for a seasonal use rate of 2.4 lb ai/A (1.2x the maximum proposed seasonal use rate). Both test substances were applied at 20 gal/A. Foliage was sampled immediately following the first, third, and fifth applications. At 28 days following the fifth application, all mature open cotton bolls were harvested and cottonseeds were removed by ginning. Samples obtained from each test plot and the control plot were pooled separately at each sampling interval. Within one hour of collection, samples were stored frozen at -27.2 to -11.7 C until shipment to the analytical laboratory (American Cyanamid Co., Princeton, NJ). Samples were shipped overnight on dry ice. Upon receipt at the analytical laboratory, samples were stored at -28.9 C until analyzed. Samples were analyzed after 40-545 days in frozen storage.

Total Radioactive Residues (TRR)

Prior to analysis, all foliage and cottonseed samples were ground with dry ice. Ground seed samples were separated by a sieve into seed meal (i.e ground cottonseed) and linters/fuzzy fibers. Total radioactive residues were determined in foliage and cottonseed samples by liquid scintillation spectroscopy (LSS) following combustion. The limit of detection was 0.01 ppm. The TRR in foliage and cottonseeds following five applications of [^{14}C]Pirate are presented in Table 1.

Residues in foliage of [pyrrole-^{14}C]- and [phenyl-14C]Pirate-treated plants were lowest after the first sampling interval and increased at each succeeding sampling interval to maximums of 122.13 and 131.79 ppm, respectively. Residues in foliage were not further characterized. Cottonseed meal and linters were analyzed for TRR separately. Apparent TRR in control samples of cottonseed and cotton foliage were <0.01 ppm.

Table 1. Total radioactive residues in/on cotton foliage, seed meal, and linters following five applications of [2-pyrrole-^{14}C]- or [phenyl-ring-labeled-^{14}C]Pirate at a seasonal rate of ~2 lb ai/A (1x).

Matrix/Sampling Interval	[^{14}C]Pirate equivalents (ppm)	
	[2-pyrrole-^{14}C]	[phenyl-^{14}C]
Foliage		
1st Application	38.49	48.30
3rd Application	93.48	102.86
5th Application	122.13	131.79
Seed Meal	0.15	0.18
Linters	0.12	0.13
Total Cottonseed	0.27	0.31

Extraction and Hydrolysis of the Residues

Ground samples of cottonseed meal and linters were homogenized in hexane. Solid resides were re-extracted with methanol (MeOH). MeOH extracts were evaporated to dryness and the dried residues were then redissolved in hexane and combined with the initial hexane extracts. Hexane-soluble residues were extracted into acetonitrile (ACN). The ACN partition step was not conducted on hexane extracts from linters of seed from [2-pyrrole-^{14}C]Pirate-treated plants because of the low oil content of the samples. The hexane extract was evaporated yielding an oil layer which was subsequently analyzed for radioactivity by LSS.

Non-extractable residues were re-extracted with aqueous 0.5% Triton X-100. The aqueous extract was then reacted with 2,2-dimethoxypropane in the presence of a small amount of hydrochloric acid (HCl) used to catalyze the reaction. The mixture was concentrated by rotary evaporation. The remaining solid residues were extracted with MeOH, and the MeOH extract evaporated to dryness and redissolved in a small volume of MeOH. The solid residues were re-extracted with MeOH:water:HCl (20:20:1, v:v:v) and the extract was adjusted to pH 5-6 with aqueous sodium hydroxide (NaOH). The neutralized extract was then reacted with 2,2-dimethoxypropane and extracted into acid; the acid extraction step was conducted only on extracts from seed meal from [2-pyrrole-^{14}C]Pirate-treated plants. The radioactivity in combined ACN extract, the aqueous Triton X-100 extract, the MeOH extract, and acid extract was determined by LSS, and then all organic extracts were combined and evaporated to dryness. The dried residues were redissolved in MeOH and analyzed by HPLC. Non-extractable radioactivity was determined by combustion/LSS.

The extraction and hydrolysis procedures described above recovered 89.2-95.8% of the TRR from cottonseed. Non-extractable residues in cottonseed fractions accounted for 8.1-11.9% of the TRR (0.01-0.02 ppm) and were not further characterized.

Characterization of the Residues

Organosoluble fractions were analyzed by reversed-phase HPLC using a C-18 column. Metabolites were detected by UV absorption at 254 nm and were co-chromatographed with a non-radiolabeled Pirate reference standard. One-minute eluate fractions were collected and radioactivity was quantified by LSS. The major component isolated from various unspecified fractions was tentatively identified as [^{14}C]Pirate. This fraction was reanalyzed by HPLC with a reference standard using a C-18 column with a different solvent system. Isolated [^{14}C]Pirate and its reference standard also were reanalyzed by HPLC using an amino reversed-phase column. In all three HPLC systems, the isolated metabolite was identified as Pirate by comparison with the retention time of its reference standard. No other reference standards were used, and no other metabolites were identified. The identity of [^{14}C]Pirate residues isolated from organosoluble extracts by HPLC analyses were also confirmed by GC/MS.

In the "Results and Discussion" section of the data submission, the petitioner states that an unspecified aliquot of Unknown-1 was incubated with ß-glucosidase and analyzed by HPLC. No method details or raw data were provided, but the petitioner stated that the results were negative, indicating that Unknown-1 is not conjugated to glucose. Further analysis of Unknown-1 by GC/MS after methylation, also indicated that this metabolite does not contain any functional hydroxyl groups. No further characterization procedures were conducted.

The distribution of radioactivity into extracts and fractions of cottonseed are presented in Table 2. The petitioner provided chromatograms and/or mass spectra for all analyses.

The parent compound, Pirate, was the major radioactive component in cottonseeds from [2-pyrrole-^{14}C]Pirate-treated plants, accounting for 54.8% of the TRR (0.08 ppm) in meal and 63.7% of the TRR (0.08 ppm) in linters. In cottonseed from [phenyl-^{14}C]Pirate-treated plants, the parent compound accounted for 73.9% of the TRR (0.13 ppm) in meal and 57.7% of the TRR (0.08 ppm) in linters. Expressed on a whole seed basis, the parent compound accounted 59.3-67.7% of the TRR (0.16-0.21 ppm). Expressed in the same manner, ^{14}C-residues in cottonseed oil and non-extractable residues accounted for <0.01-0.01 ppm (≤3.2% TRR) and 0.03 ppm (9.7-11.1% TRR), respectively.

An unidentified polar metabolite (designated Unknown-1) accounted for 4.7-7.1% of the TRR (0.01 ppm) in meal and 3.4-3.5% of the TRR (<0.01 ppm) in linters. Characterization of Unknown-1 indicated that it was not conjugated to glucose and it did not contain a functional hydroxyl group. Numerous minor radioactive peaks (≤0.01 ppm) also were observed in organosoluble extracts of meal and linters. The registrant made several attempts to identify these peaks using reference standards of N-dealkylation, dehalogenation, hydroxylation, and carboxylation analogs of the parent compound; however, none of these peaks corresponded to these reference standards. No raw data were provided for these analyses. Radiochromatograms were presented without any supporting raw data for radioanalysis. Therefore, the amount of radioactivity contained in each individual unknown peak could not be independently determined.

For purposes of this EUP and temporary tolerance petition, the nature of the residue is adequately understood. The residue to be regulated is the parent compound Pirate.

For establishment of a permanent tolerance the nature of the residue is not adequately understood. Raw data are needed showing the amount of radioactivity in individual unknown peaks detected in organosoluble extracts of cottonseed meal and linters. In addition, data must be submitted demonstrating the stability of ^{14}C-residues in samples stored for 545 days (~18 months). The petitioner indicated that the storage intervals for cottonseed prior to analysis ranged from 40 to 501

days. HPLC chromatograms showing that 40- and 501-day metabolite profiles are similar would fulfill this requirement.

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Table 2. Distribution of radioactivity in **cottonseeds** following five foliar applications of [2-pyrrole-^{14}C]Pirate or [phenyl-ring-^{14}C]Pirate at ~1x.

Matrix	Fraction	%TRR	ppm	Characterization/Identification
[2-pyrrole-^{14}C]Pirate				
Seed meal (0.15 ppm)	Hexane	37.4	0.06	Not analyzed separately.
	MeOH I	34.2	0.05	Not analyzed separately.
	Surfactant, MeOH, & MeOH:water:HCl	19.5	0.03	Not analyzed separately.
	Total Organosoluble [a]	91.1	0.14	HPLC analysis isolated Pirate (54.8% TRR, 0.08 ppm), Unknown-1 (7.1% TRR, 0.01 ppm), and 15 other unknowns (collectively accounting for 19.2% TRR, 0.03 ppm). Total Characterized and identified = 81.1% TRR (0.12 ppm)
	Oil	2.2	<0.01	NA (not further analyzed).
	Non-extractable	11.9	0.02	NA.
Linters (0.12 ppm)	Hexane[b]	35.4	0.04	Not analyzed separately.
	MeOH I	36.8	0.04	Not analyzed separately.
	Surfactant, MeOH, & MeOH:water:HCl	22.5	0.03	Not analyzed separately.
	Total Organosoluble [a]	94.7	0.11	HPLC analysis isolated Pirate (63.7% TRR, 0.08 ppm), Unknown-1 (3.4% TRR, <0.01 ppm), and 22 unknowns (collectively accounting for 24.3% TRR, 0.03 ppm). Total Identified/Characterized = 91.4% TRR (<0.12 ppm)
	Oil	--[d]	--	NA.
	Non-extractable	8.1	0.01	NA.
[phenyl-ring-^{14}C]Pirate				
Seed meal (0.18 ppm)	Hexane	52.7	0.09	Not analyzed separately.
	MeOH I	31.6	0.06	Not analyzed separately.
	Surfactant & MeOH [c]	11.5	0.02	Not analyzed separately.
	Total Organosoluble [a]	95.8	0.17	HPLC analysis isolated Pirate (73.9% TRR, 0.13 ppm), Unknown 1 (4.7% TRR, 0.01 ppm), and 16 other unknowns (collectively accounting for 9.4% TRR, 0.02 ppm). Total Identified/Characterized = 88.0% TRR (0.16 ppm)
	Oil	3.6	<0.01	NA.
	Non-extractable	9.4	0.02	NA.

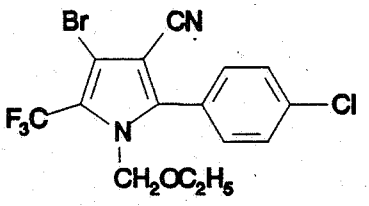
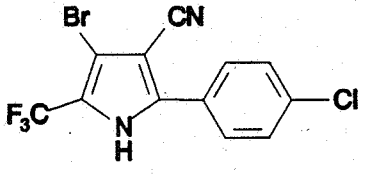
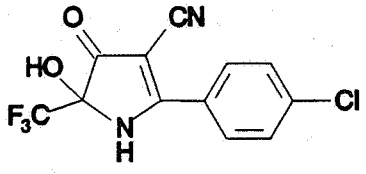
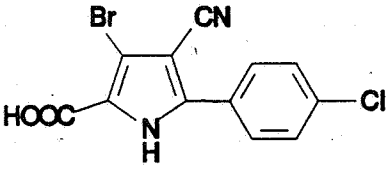
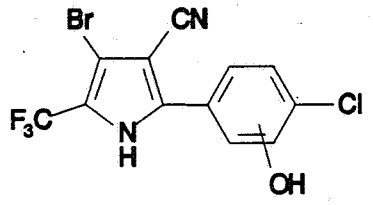
(continued; footnotes follow)

Table 2 *(continued)*.

Matrix	Fraction	%TRR	ppm	Characterization/Identification
Linters (0.13 ppm)	Hexane	44.0	0.06	Not analyzed separately.
	MeOH I	33.4	0.04	Not analyzed separately.
	Surfactant, MeOH, & MeOH:water:HCl	11.8	0.02	Not analyzed separately.
	Total Organosoluble [a]	89.2	0.12	HPLC analysis isolated Pirate (57.7% TRR, 0.08 ppm), Unknown 1 (3.5% TRR, <0.01 ppm), and 22 other unknowns (collectively accounting for 21.2% TRR, 0.03 ppm). Total Identified/Characterized = 82.4% TRR (<0.12 ppm)
	Oil	1.8	<0.01	NA.
	Non-extractable	8.8	0.01	NA.

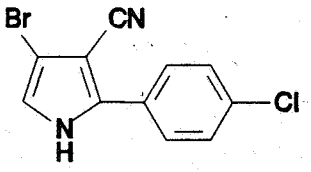
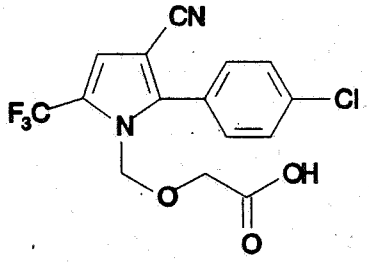
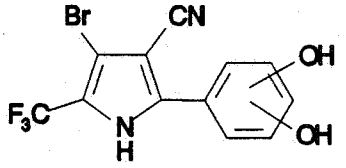
[a] Extracts were pooled into a total organosoluble fraction prior to HPLC analysis.
[b] The ACN partition step was not conducted on hexane extracts from Fraction II of seed from [2-pyrrole-^{14}C]Pirate-treated plants because of the low oil content of the samples.
[c] Cottonseed meal from [phenyl-ring-^{14}C]Pirate-treated plants was not fractionated using the MeOH:water:HCl step.
[d] The oil fraction was not isolated from [2-pyrrole-^{14}C]Pirate-treated plants.

Figure 1. Pirate and its metabolites in plants and animals (MRIDs 42770234, 42770235, and 42770236).

Code	Chemical Name Structure	Substrate Common Name (Chemical Code)
I.	4-bromo-2-(p-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-1-M-pyrrole-3-carbonitrile 	cottonseed meal goat milk, liver, kidney, fat, and muscle hen egg, liver, kidney, skin, and muscle **Pirate (CL 303,630)**
II.	4-bromo-2-(p-chlorophenyl)-5-(trifluoromethyl)-pyrrole-3-carbonitrile 	goat milk, liver, kidney, fat, and muscle hen egg, liver, kidney, skin, and muscle **(CL 303,268)**
III.	2-(p-chlorophenyl)-5-hydroxy-4-oxo-5-(trifluoromethyl)-2-pyrrolidine-3-carbonitrile 	goat milk, liver, kidney, fat, and muscle hen liver and kidney **(CL 325,195)**
IV.	3-bromo-5-(p-chlorophenyl)-4-cyano-pyrrole-2-carboxylic acid 	goat milk [a], liver [a], kidney [a], fat [a], and muscle [a] hen egg, liver, and kidney **(CL 322,250)**
V.	4-bromo-2-(4-chloro-*ar*-hydroxyphenyl)-5-(trifluoromethyl)-pyrrole-3-carbonitrile 	goat milk, liver, kidney, fat, and muscle hen liver and kidney **(AC 8944-45)**

(continued; footnotes follow)

Figure 1 *(continued)*.

Code	Chemical Name Structure	Substrate Common Name (Chemical Code)
VI.	4-bromo-2-(p-chlorophenyl)-pyrrole-3-carbonitrile	
		goat milk *, liver *, kidney *, fat *, and muscle * hen egg, liver, kidney, skin, and muscle **(AC 8508-33-B-1)**
VII.	[3-bromo-5-(p-chlorophenyl)-4-cyano-2-(trifluoromethyl)pyrrole-1-yl]methoxyacetic acid	
		hen egg, liver, kidney, and muscle **(AC 8508-50-C)**
VIII.	4-bromo-2-(dihydroxyphenyl)-5-(trifluoromethyl)-pyrrole-3-carbonitrile	
		goat milk *, liver *, kidney *, fat *, muscle *, hen kidney **(AC 8508-78-BB)**

* Identification not confirmed by a second method.

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Qualitative Nature of the Residue in Animals

Goats

American Cyanamid (1993; MRID 42770235) submitted data depicting the metabolism of [^{14}C]Pirate in lactating goats. The in-life and analytical phases of the study were conducted by American Cyanamid Company (Princeton, NJ). A total of five goats were used in the study. One goat received placebo capsules and four goats, two per test substance, were dosed orally once a day for seven consecutive days, with ca. 6 mg (low dose) and 30 mg (high dose) of either uniformly ring-labeled [phenyl-^{14}C]Pirate (radiochemical purity 97.3%; specific activity of 4.35 μCi/mg) or [2-pyrrole ^{14}C]Pirate (radiochemical purity 97.4%; specific activity of 4.36 μCi/mg). One remaining goat served as a control. The low and high doses represented a daily feeding level of 3.0 and 17.9 ppm for [phenyl-^{14}C]Pirate and 3.16 ppm and 16.4 ppm for [pyrrole-^{14}C]Pirate.

Based on a worst case diet consisting of 25% cottonseeds, 15% cottonseed meal, and 15% cottonseed hulls plus soapstock (each at the 0.5 ppm proposed tolerance for cottonseed) and 40% alfalfa forage (at 0 ppm), the maximum daily dietary intake of Pirate by ruminants is estimated to be (.25 + .15 + .15 x 0.5 = .275/89 % dry matter) or 0.31 ppm. Therefore, the low and high doses of [^{14}C] Pirate corresponded to a maximum daily dietary burden of ca. 10x and 58x, respectively.

Milk samples were collected twice daily. Approximately 24 hours after the last dose, the test animals were sacrificed, and kidney, liver, muscle (leg and tenderloin combined), and omental fat samples were collected. All samples were frozen (at unspecified temperature) immediately after sampling. Dates of TRR analysis and extraction of tissues were provided; these data indicate that extractions were completed within ca. 5 months and analyses were completed ca. 8 months (from dates on MS chromatograms) after sample collection. No other storage stability data were provided.

Total Radioactive Residues (TRR)

Triplicate milk samples were analyzed directly by LSS and tissue subsamples were analyzed by LSS following combustion. The limit of detection for the radioassays was 0.01 ppm. Sample calculations and raw data were submitted. Both [phenyl-^{14}C]- and [pyrrole-^{14}C]-labeled samples showed similar distribution patterns of radioactive residues in tissues and milk (Table 3). During the testing period, radioactive residues in milk samples from the low dose group remained at 0.02 ppm. In the high dose group, the TRR in milk samples increased during the test period from 0.03 ppm to 0.07 ppm. Radioactive residues were highest in the high dose group with the largest residue found in liver (1.45-1.46 ppm); the ^{14}C-residues in other tissues from the high dose group ranged from 0.03-0.05 ppm in muscle to 0.62-0.94 ppm in kidney.

Table 3. Total radioactive residues (TRR) in milk and tissues from lactating goats dosed with [phenyl-^{14}C]- or [pyrrole-^{14}C]Pirate at 10x and 58x for seven consecutive days.

Matrix	TRR (Pirate equivalents, ppm)			
	Low dose (10X)		High Dose (58X)	
	[phenyl-^{14}C]	[pyrrole-^{14}C]	[phenyl-^{14}C]	[pyrrole-^{14}C]
Milk Day 1	0.01	0.02	0.03	0.06
Day 2	0.02	0.02	0.04	0.06
Day 3	0.02	0.02	0.03	0.06
Day 4	0.02	0.02	0.04	0.07
Day 5	0.02	0.02	0.05	0.06
Day 6	0.02	0.02	0.04	0.06
Day 7	0.02	0.02	0.04	0.07

Liver	0.51	0.51	1.46	1.45
Kidney	0.40	0.37	0.94	0.62
Fat	0.10	0.07	0.11	0.24
Muscle	0.02	0.02	0.03	0.05

Extraction of Residues

Milk samples from goats dosed with [phenyl-^{14}C] or [pyrrole-^{14}C]Pirate at 3 ppm (low dose) were not analyzed and the tissue solids were not hydrolyzed. Additionally, the data from the high dose samples were comparable to the available data from low dose tissues. Therefore, the data from the low dose samples are not presented in this report.

Kidney, liver, and muscle were extracted three times with MeOH. For each sample, the resulting extracts were combined, concentrated, and partitioned with ACN:MeOH:water (8:1:1; v:v:v) and hexane. The ACN:MeOH:water and hexane fractions were concentrated and analyzed by HPLC. Liver and kidney solids were acid and base hydrolyzed as described below.

Fat was extracted with ACN and partitioned with hexane. Both ACN and hexane fractions were concentrated and analyzed by HPLC.

Milk (7-day) solids were precipitated with methylene chloride (CH_2Cl_2):acetone (1:12; v:v). The supernatant was refrigerated overnight and filtered to remove additional milk solids. The filtrate was extracted three times with CH_2Cl_2, and the organic fractions were pooled, concentrated, and redissolved in hexane. The hexane fraction was partitioned with ACN and the resulting hexane and ACN fractions were concentrated and analyzed by HPLC.

Hydrolysis of Residues

Subsamples of non-extractable liver and kidney fractions were sequentially hydrolysed and filtered as follows: (i) sonicated for 3 minutes in MeOH:water:concentrated HCl (40:50:2; v:v:v), (ii) extracted for 1 hour in MeOH:water:HCl with mechanical shaking, (iii) refluxed for 1 hour in 1 N HCl, (iv) refluxed overnight in 1 N HCl, and (v) stirred overnight at ambient temperature in 1 N sodium hydroxide. Additional subsamples were refluxed overnight in 1 N HCl followed by overnight incubation in 1 N sodium hydroxide at ambient temperature. Selected fractions of MeOH:water:HCl and acid hydrolysates were concentrated and analyzed by HPLC; the base hydrolysates were not analyzed by HPLC.

Control samples of milk and tissue fortified with [phenyl-14C]- or [pyrrole-^{14}C]Pirate were extracted and analyzed. The data indicated that [^{14}C]Pirate is stable through the extraction and 1 hour HCl reflux procedures. However, data from the 1 hour and overnight HCl hydrolysis procedures of non-extractable residues from liver samples fortified with [pyrrole-^{14}C]Pirate indicated partial dealkylation of Pirate producing CL 303,268 [4-bromo-2-(p-chlorophenyl)-5-(trifluoromethyl)-pyrrole-3-carbonitrile].

The molecular structures and chemical names of Pirate and suspected metabolites are presented in Figure 1. The distribution of ^{14}C-activity in milk and tissue fractions from goats dosed with [^{14}C]Pirate at 17.9 ppm with [phenyl-^{14}C]Pirate or 16.4 ppm of [pyrrole-^{14}C]Pirate (high dose) are summarized in Table 4.

Table 4. Distribution of TRR in milk and tissues from lactating goats dosed with [phenyl-^{14}C]- or [pyrrole-^{14}C]Pirate at 58x for seven consecutive days.

Substrate	Fraction	Pirate equivalents		Characterization/Identification
		%TRR	ppm	
Milk [phenyl-^{14}C] (0.04 ppm)	MeOH	87.7	0.04	Solvent partitioned.
	Hexane	2.0	<0.01	Not analyzed = N/A
	ACN/MeOH/Water	77.3	0.03	Pirate (24.7% TRR, <0.01 ppm), CL 303,268 (8.4% TRR, <0.01 ppm), CL 325,195 (8.4% TRR, <0.01 ppm), and AC 8944-45 (1.1% TRR, <0.01 ppm), were identified by HPLC/2-D TLC. Pirate was confirmed by GC/MS. Metabolites M-3 (3.0% TRR, <0.01 ppm), M-5A (1.6% TRR, <0.01 ppm), M-6 (1.7% TRR, <0.01 ppm), M-7A (0.8% TRR, <0.01 ppm), and four Unknowns (22.8% TRR, <0.04 ppm) were resolved by HPLC.
	Aqueous	8.4	<0.01	N/A
	Non-extractable	12.3	<0.01	N/A
Milk [pyrrole-^{14}C] (0.07 ppm)	MeOH	91.7	0.06	Solvent partitioned.
	Hexane	1.5	<0.01	N/A
	ACN/MeOH/Water	87.5	0.06	Pirate (68.4% TRR, 0.05 ppm), CL 303,268 (5.5% TRR, <0.01 ppm), CL 325,195 (1.8% TRR, <0.01 ppm), and AC 8944-45 (0.4% TRR, <0.01 ppm) were identified by HPLC/2-D TLC. Metabolites M-3 (1.7% TRR, <0.01 ppm), M-5A (<0.1% TRR, <0.01 ppm), M-6 (0.2% TRR, <0.01 ppm), M-7A (1.1% TRR, <0.01 ppm), and three Unknowns (6.4% TRR, <0.03 ppm) were resolved by HPLC.
	Aqueous	2.7	<0.01	N/A
	Non-extractable	8.3	<0.01	N/A
Liver, [phenyl-^{14}C] (1.46 ppm)	MeOH	20.4	0.29	Solvent partitioned.
	Hexane	4.8	0.06	Resolved pirate and CL 325,195 by HPLC.
	ACN/MeOH/Water	15.6	0.23	Pirate (2.3% TRR, 0.03 ppm), CL 303,268 (2.6% TRR, 0.03 ppm), CL 325,195 (3.9% TRR, 0.06 ppm), and AC 8944-45 (2.3% TRR, 0.04 ppm), were identified by HPLC/2-D TLC. Metabolites M-3 (1.3% TRR, 0.02 ppm), M-5A (0.6% TRR, <0.01 ppm), M-6 (0.4% TRR, <0.01 ppm), M-7A (0.8% TRR, <0.01 ppm), and three Unknowns (1.0% TRR, <0.03 ppm) were resolved by HPLC [a,b].
	Non-extractable	79.6	1.16	Acid and base hydrolysed.
	HCl extract	40.0	0.58	N/A
	NaOH extract	33.3	0.49	N/A
	Non-extractable	7.5	0.11	N/A

(continued; footnotes follow)

Table 4 *(continued)*.

Substrate	Fraction	Pirate equivalents		Characterization/Identification
		%TRR	ppm	
Liver, [pyrrole-^{14}C] (1.45 ppm)	MeOH	19.0	0.28	Solvent partitioned.
	Hexane	4.0	0.06	Resolved pirate and CL 325,195 by HPLC.
	ACN/MeOH/Water	15.0	0.22	Pirate (2.8% TRR, 0.04 ppm), CL 303,268 (1.5% TRR, 0.02 ppm), CL 325,195 (13.5% TRR, 0.20 ppm), and AC 8944-45 (2.7% TRR, 0.04 ppm) were identified by HPLC/2-D TLC. Metabolites M-3 (9.9% TRR, 0.14 ppm), M-5A (3.3% TRR, 0.07 ppm), M-6 (1.0% TRR, 0.01 ppm), M-7A (2.8% TRR, 0.04 ppm), and three Unknowns (15.5% TRR, 0.23 ppm) were resolved by HPLC *.
	Non-extractable	81.0	1.17	Acid/base hydrolysed.
	Sonic disruption	1.4	0.02	N/A
	MeOH/Water/HCL extract	4.1	0.06	Resolved pirate, CL 325,195, CL 322,250, and AC 8944-45 by HPLC.
	HCl extract (1 hour)	26.2	0.38	Resolved CL 325,195, CL 322,250, AC 8805-31-2-B, and three Unknowns by HPLC.
	HCl extract (overnight)	22.8	0.33	Resolved CL 325,195, CL 322,250, and 3 Unknowns by HPLC.
	NaOH extract	10.3	0.15	N/A
	Non-extractable	16.6	0.24	N/A
Kidney [phenyl-^{14}C] (0.94 ppm)	MeOH	16.1	0.15	Solvent partitioned.
	Hexane	9.9	0.09	Resolved pirate, CL 325,195, CL 322,250, AC 8944-45, AC 8508-31-2-B, and two Unknowns by HPLC.
	ACN/MeOH/Water	6.2	0.06	Pirate (8.3% TRR, 0.08 ppm), CL 303,268 (0.2% TRR, 0.02 ppm), CL 325,195 (4.3% TRR, 0.04 ppm), and AC 8944-45 (0.3% TRR, <0.01 ppm) were identified by HPLC/2-D TLC. Metabolites M-3 (2.7% TRR, 0.03 ppm), M-5A (2.0% TRR, 0.02 ppm), M-6 (0.2% TRR, <0.01 ppm), M-7A (1.3% TRR, 0.01 ppm), and five Unknowns (6.8% TRR, <0.09 ppm) were resolved by HPLC *.
	Non-extractable	83.9	0.79	Acid/base hydrolysed.
	Sonic disruption	2.1	0.02	N/A
	MeOH/Water/HCL extract	11.7	0.11	Resolved pirate and two Unknowns by HPLC.
	HCL extract (1 hour)	13.8	0.14	N/A
	HCl extract (overnight)	7.4	0.07	Resolved three Unknowns by HPLC.
	NaOH extract	39.4	0.37	N/A
	Non-extractable	8.5	0.08	N/A

(continued; footnotes follow)

Table 4 *(continued)*.

Substrate	Fraction	Pirate equivalents		Characterization/Identification
		%TRR	ppm	
Kidney, [pyrrole-^{14}C] (0.62 ppm)	MeOH	16.2	0.10	Solvent partitioned.
	Hexane	1.7	0.01	Resolved pirate, AC 8944-45, CL 325,195, and three Unknowns by HPLC.
	ACN/MeOH/Water	14.5	0.09	Pirate (9.6% TRR, 0.06 ppm), CL 303,268 (0.6% TRR, <0.01 ppm), CL 325,195 (0.4% TRR, <0.01 ppm), and AC 8944-45 (0.3% TRR, <0.01 ppm) were identified by HPLC/2-D TLC. Metabolites M-3 (0.9% TRR, <0.01 ppm), M-5A (0.1% TRR, <0.01 ppm), M-7A (0.5% TRR, <0.01 ppm), and four Unknowns (<1.4% TRR, <0.04 ppm) were resolved by HPLC [a].
	Non-extractable	83.8	0.52	Acid/base hydrolysed.
	HCl extract (overnight)	41.3	0.26	N/A
	NaOH extract	38.2	0.24	N/A
	Non-extractable	4.4	0.03	N/A
Fat, [phenyl-^{14}C] 0.11 ppm	MeOH	87.7	0.09	Solvent partitioned.
	Hexane	8.6	<0.1	N/A
	ACN/MeOH/Water	79.1	0.09	Pirate (46.9% TRR, 0.05 ppm), CL 303,268 (19.3% TRR, 0.02 ppm), CL 325,195 (0.8% TRR, <0.01 ppm), and AC 8944-45 (3.6% TRR, <0.01 ppm) were identified by HPLC/2-D TLC. Metabolites M-3 (0.6% TRR, <0.01 ppm), M-5A (0.4% TRR, <0.01 ppm), M-6 (0.7% TRR, <0.01 ppm), M-7A (9.4% TRR, <0.01 ppm), and four Unknowns (1.8% TRR, <0.04 ppm) were resolved by HPLC.
	Non-extractable	12.3	0.01	N/A
Fat, [pyrrole-^{14}C] 0.24 ppm	MeOH	95.0	0.23	Solvent partitioned.
	Hexane	1.9	<0.01	N/A
	ACN/MeOH/Water	93.1	0.22	Pirate (78.0% TRR, 0.19 ppm), CL 303,268 (9.9% TRR, 0.02 ppm), CL 325,195 (0.7% TRR, <0.01 ppm), and AC 8944-45 (0.9% TRR, <0.01 ppm) were identified by HPLC/2-D TLC. Metabolites M-3 (0.2% TRR, <0.01 ppm), M-5A (0.3% TRR, <0.01 ppm), M-6 (<0.1% TRR, <0.01 ppm), M-7A (2.6% TRR, <0.01 ppm), and three Unknowns (0.9% TRR, <0.03 ppm) were resolved by HPLC.
	Non-extractable	5.0	0.01	N/A
Muscle, [phenyl-^{14}C] 0.03 ppm[c]	MeOH	32.0	<0.01	Pirate (52.0% TRR, 0.01 ppm), CL 303,268 (1.9% TRR, <0.01 ppm), CL 325,195 (0.3% TRR, <0.01 ppm), and AC 8944-45 (0.1% TRR, <0.01 ppm) were identified by HPLC/2-D TLC. Metabolites M-3 (0.9% TRR, <0.01 ppm), M-6 (0.1% TRR, <0.01 ppm), M-7A (0.3% TRR, <0.01 ppm), and two Unknowns (0.4% TRR, <0.02 ppm) were resolved by HPLC.
	Non-extractable	68.0	0.02	N/A

(continued; footnotes follow)

Table 4 *(continued)*.

Substrate	Fraction	Pirate equivalents		Characterization/Identification
		%TRR	ppm	
Muscle [pyrrole-^{14}C] 0.05 ppm	MeOH	48.0	0.02	Solvent partitioned.
	Hexane	10.2	<0.01	N/A
	ACN/MeOH/Water	37.8	0.02	Pirate (28.7% TRR, 0.01 ppm), CL 303,268 (2.5% TRR, <0.01 ppm), CL 325,195 (0.6% TRR, <0.01 ppm), and AC 8944-45 (0.3% TRR, <0.01 ppm) were identified by HPLC/2-D TLC. Metabolites M-5A (0.5% TRR, <0.01 ppm), M-7A (0.2% TRR, <0.01 ppm), and three Unknowns (<2.0% TRR, <0.03 ppm) were resolved by HPLC.
	Non-extractable	52.0	0.03	N/A

[a] Includes metabolites (% TRR and ppm) characterized/identified from other extracts.
[b] CL 325,195 identified from goat urine was confirmed by LC/MS and GC/MS and AC 8944-45 identified from goat feces was confirmed by LC/MS; both of these metabolites were compared with that isolated from goat liver.
[c] The methanol extract was not partitioned into hexane and ACN.

Characterization of Residues

Milk and tissue organic fractions were analyzed by reversed-phase HPLC using a water:ACN (1:9; v:v) solvent system containing 1% glacial acetic acid (v:v). Reference compounds were visualized using a UV detector at 254 or 260 nm and radioactivity in the HPLC fractions were quantified by LSS.

For confirmation of metabolites resolved by HPLC, 1-D or 2-D TLC analyses were performed on silica gel plates using several solvent systems. The petitioner stated that liver fractions were analyzed using reversed-phase silica gel TLC with a MeOH:water:acetic acid (180:20:0.5; v:v:v) solvent system; however, no chromatograms or analytical data were submitted. The reference standards on TLC plates were visualized under UV (254 nm) and the radioactive zones on TLC plates were visualized by autoradiography and quantified by scraping/LSS. Radioactive metabolites were confirmed by cochromatography with reference standards. Structural confirmation of the parent compound and selected metabolites were performed by liquid chromatography/mass spectroscopy (LC/MS) or GC/MS. Samples of representative chromatograms were submitted.

A summary of the components characterized/identified from milk and tissue samples from goats dosed with [phenyl-^{14}C]- or [pyrrole-^{14}C]Pirate at 58x is presented in Tables 5 and 6.

Table 5. Characterization/identification of radioactive residues in lactating goats dosed with [phenyl-^{14}C] Pirate at 58x for seven consecutive days.

Metabolite/Extract	Milk		Liver		Kidney		Fat		Muscle	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	PPM
CL 303,630 (Pirate)	24.7	<0.01	2.3	0.03	8.3	0.08	46.9	0.05	52.0	0.01
CL 303,268 (M-8)	8.4	<0.01	2.6	0.03	0.2	<0.01	19.3	0.02	1.9	<0.01
CL 325,195 (M-5)	8.4	<0.01	3.9	0.06	4.3	0.04	0.8	<0.01	0.3	<0.01
AC 8944-45 (M-7)	1.1	<0.01	2.3	0.04	0.3	<0.01	3.6	<0.01	0.1	<0.01
AC 8508-78-BA/BB (M-3) [a,b]	3.0	<0.01	1.3	0.02	2.7	0.03	0.6	<0.01	0.9	<0.01
CL 322,250 (M-5A) [b]	1.6	<0.01	0.6	<0.01	2.0	0.02	0.4	<0.01	<0.1	<0.01
AC 8805-31-2-B (M-6) [b]	1.7	<0.01	0.4	<0.01	0.2	<0.01	0.7	<0.01	0.1	<0.01
AC 8508-33-B-1 (M-7A) [b]	0.8	<0.01	0.8	<0.01	1.3	<0.01	9.4	<0.01	0.3	<0.01
Total Identified	49.7	<0.08	14.2	<0.21	19.3	<0.21	81.7	<0.13	<55.7	<0.08
Unknown 1	19.2	<0.01	0.1	<0.01	0.3	<0.01	0.7	<0.01	0.2	<0.01
Unknown 2	0.8	<0.01	0.3	<0.01	0.3	<0.01	0.2	<0.01	--	--
Unknown 4	2.6	<0.01	0.6	<0.01	5.2	0.05	0.8	<0.01	0.2	<0.01
Unknown 10	--	--	--	--	0.9	<0.01	--	--	--	--
Unknown 11	0.2	<0.01	--	--	0.1	<0.01	0.1	<0.01	--	--
Hexane extract [c]	2.0	<0.01	--	--	--	--	8.6	<0.01	--	--
Aqueous extract [c]	8.4	<0.01	--	--	--	--	--	--	--	--
Sonicated extract [c]	--	--	--	--	2.1	0.02	--	--	--	--
MeOH/Water/HCL extract	--	--	--	--	[c]	[c]	--	--	--	--
HCL extract (1 hour) [c]	--	--	--	--	13.8	0.14	--	--	--	--
HCL extract (Overnight) [c]	--	--	40.0	0.58	--	--	--	--	--	--
NaOH extract [c]	--	--	33.3	0.49	39.4	0.37	--	--	--	--
Total Characterized	**82.9**	**<0.14**	**88.5**	**<1.31**	**81.4**	**<0.83**	**92.1**	**<0.18**	**56.1**	**<0.10**
Non-extractable	12.3	<0.01	7.5	0.11	8.5	0.08	12.3	0.01	68.0	0.02

[a] Identified as a mixture of AC 8508-78-BA (sulfate conjugate of CL 322,250) and AC 8508-78-BB.
[b] Identification not confirmed by a second method.
[c] Selected extracts analyzed by HPLC and the metabolite TRRs were added to the respective identified and characterized components.

Table 6. Summary of characterization/identification of radioactive residues in lactating goats dosed with [pyrrole-^{14}C] Pirate at 58x for seven consecutive days.

Metabolite/Extract	Milk		Liver		Kidney		Fat		Muscle	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	PPM
CL 303,630 (Pirate)	68.4	0.05	2.8	0.04	9.6	0.06	78.0	0.19	28.7	0.01
CL 303,268 (M-8)	5.5	<0.01	1.5	0.02	0.6	0.02	9.9	0.02	2.5	<0.01
CL 325,195 (M-5)	1.8	<0.01	13.5	0.20	0.4	<0.01	0.7	<0.01	0.6	<0.01
AC 8944-45 (M-7)	0.4	<0.01	2.7	0.04	0.3	<0.01	0.9	<0.01	0.3	<0.01
AC 8508-78-BA/BB (M-3) [a,b]	1.7	<0.01	9.9	0.14	0.9	<0.01	0.2	<0.01	<0.1	<0.01
CL 322,250 (M-5A) [b]	<0.1	<0.01	3.3	0.07	0.1	<0.01	0.3	<0.01	0.5	<0.01
AC 8805-31-2-B (M-6) [b]	0.2	<0.01	1.0	0.01	<0.1	<0.01	<0.1	<0.01	--	--
AC 8508-33-B-1 (M-7A) [b]	1.1	<0.01	2.8	0.04	0.5	<0.01	2.6	<0.01	0.2	<0.01
Total Identified	<79.2	<0.12	37.5	0.56	<12.5	<0.14	92.7	<0.23	32.9	<0.07
Unknown 1	3.1	<0.01	3.4	0.05	<0.1	<0.01	0.2	<0.01	0.2	<0.01
Unknown 2	0.9	<0.01	5.5	0.08	0.2	<0.01	<0.1	<0.01	<0.1	<0.01
Unknown 4	2.4	<0.01	6.6	0.10	0.9	<0.01	0.5	<0.01	1.7	<0.01
Unknown 10	--	--	--	--	0.2	<0.01	--	--	--	--
Unknown 11	--	--	--	--	--	--	0.2	<0.01	--	--
Hexane extract [c]	1.5	<0.01	--	--	--	--	1.9	<0.01	10.2	<0.01
Aqueous extract [c]	2.7	<0.01	--	--	--	--	--	--	--	--
Sonicated extract [c]	--	--	1.4	0.02	--	--	--	--	--	--
MeOH/Water/HCl extract	--	--	[c]	[c]	--	--	--	--	--	--
HCL extract (1 hour)	--	--	[c]	[c]	--	--	--	--	--	--
HCL extract (Overnight) [c]	--	--	--	--	41.3	0.26	--	--	--	--
NaOH extract [c]	--	--	10.3	0.15	38.2	0.24	--	--	--	--
Total Characterized	**89.8**	**0.17**	**64.7**	**0.96**	**93.5**	**0.68**	**95.6**	**0.32**	**45.1**	**0.11**
Non-extractable	8.3	<0.01	16.6	0.24	4.4	0.03	5.0	0.01	52.0	0.03

[a] Identified as a mixture of AC 8508-78-BA (sulfate conjugate of CL 322,250) and AC 8508-78-BB.
[b] Identification not confirmed by a second method.
[c] Selected extracts analyzed by HPLC and the metabolites TRRs were added to the respective identified and characterized components.

Summary of goat metabolism: Lactating goats were orally dosed with [phenyl-^{14}C]- and [pyrrole-^{14}C]Pirate at ca. 10x and 58x the proposed maximum daily dietary burden. The distribution of TRR in milk and tissues from both groups were similar. In the high dose group, the TRR in milk increased from 0.03 to 0.07 ppm by day 7. Radioactive residues were highest in liver (1.45-1.46 ppm) and ranged from 0.03-0.05 ppm in muscle to 0.62-0.94 ppm in kidney.

Approximately 79% of the administered radioactivity in Pirate was excreted by lactating goats in the urine and feces. The parent compound and eight metabolites were detected in milk and tissues; two metabolites (AC 8508-78-BA/BB) were identified as a mixture. The total identified/characterized ^{14}C-residues were: ≥83% TRR (milk), ≥65% TRR (liver), ≥81% TRR (kidney), ≥92% TRR (fat), and ≥45% TRR (muscle). Pirate per se was the major metabolite detected in milk (24.7-68.4% TRR), kidney (8.3-9.6% TRR), fat (46.9-78.0% TRR), and muscle (28.7-52.0% TRR). The major metabolite detected in liver was CL 325,195 (3.9-13.5% TRR, 0.06-0.20 ppm). Unknowns 1, 2, 4, 10, and 11, accounting for <0.1-19.2% of the TRR (<0.01-0.1 ppm), were also detected. Non-extractable residues in milk and tissue samples accounted for 4.4-68.0% of the TRR (<0.01-0.24 ppm). Non-extracted ^{14}C-residues from muscle samples (52.0-68.0% TRR, 0.02-0.03 ppm) were not further examined.

The data indicate that Pirate is metabolized in lactating goats by N-dealkylation, dehalogenation, ring hydroxylation, and/or oxidation of the terminal alkyl group. Pirate is N-dealkylated to form CL 303,268, which undergoes ring hydroxylation producing CL 325,195, AC 8944-45, and AC 8508-78-BB. The terminal alkyl group of Pirate is oxidized and dehalogenated to produce AC 8805-31-2-B. All the identified metabolites contain ^{14}C-labels from the [phenyl-^{14}C]- and [pyrrole-^{14}C]Pirate, indicating no fragmentation of the phenyl and pyrrole rings during the metabolism of Pirate in goats. The petitioner's proposed metabolic pathway for Pirate in poultry and ruminants is included as Attachment 2.

Laying Hens

American Cyanamid (1993; MRID 42770236) submitted data depicting the metabolism of uniformly ring-labeled [phenyl-^{14}C]Pirate and [2-pyrrole-^{14}C]Pirate in laying hens. The in-life and analytical phases of the study were conducted by American Cyanamid Company (Princeton, NJ). Four groups of laying hens, 8 per group, were dosed with either [phenyl-^{14}C]Pirate (specific activity of 4.21 μCi/mg; radiochemical purity 98.6%) or [pyrrole-^{14}C]Pirate (specific activity 4.16 μCi/mg; radiochemical purity 97.8%) at high and low doses for seven consecutive days. A fifth group of hens was used as a control. Based on a daily feed consumption, the petitioner estimated that the low and high doses were equivalent to 3.02-3.10 ppm and 14.42-15.04 ppm of Pirate in the diet, respectively.

Based on a worst case poultry diet consisting of 20% cottonseed meal (at 0.5 ppm proposed tolerance) and 80% grain (at 0 ppm), the maximum daily dietary intake of Pirate by poultry is estimated to be (.20 x 0.5 = .10/89% dry matter) or 0.11 ppm. Therefore, the low and high doses of [^{14}C]Pirate corresponded to a maximum daily dietary burden of 27.5-28x (ca. 28x) and 131-137x (ca. 134x).

Eggs and excreta were collected daily. The hens were sacrificed within 22 hours of the final dose, and liver, muscle, kidney, and skin plus underlying fat were collected. The eggs, excreta, and tissues from each treatment group were pooled to form composite samples. Egg and tissue samples were frozen immediately upon collection and stored for 8-288 days (9.5 months) at -20 C until analyzed. No other storage stability data were provided.

Total Radioactive Residues (TRR)

Liver, kidney, muscle, and skin/fat samples were ground in dry ice, and eggs from each days collection were homogenized. Triplicate aliquots of egg and tissue homogenates were analyzed for total radioactive residues by LSS following combustion. The detection limit for the radioassays was 0.01 ppm for all tissues, except skin, which had a detection limit of 0.02 ppm. Equations used to calculate the TRRs in ppm equivalents were provided. The TRRs in egg and tissue samples from laying hens dosed with [phenyl-^{14}C]- and [pyrrole-^{14}C]Pirate are presented in Table 7. In high dose hens, the TRR in eggs increased during the test period to 0.37-0.42 ppm. Radioactive residues were highest in liver (1.25-1.31 ppm); the 14C-residues in other tissues ranged from 0.02 ppm in muscle to 0.37-0.52 ppm in kidney. Both [phenyl-^{14}C]- and [pyrrole-^{14}C]-labeled samples showed similar distribution patterns of radioactive residues in tissues and eggs. Approximately 78-94% of the low dose ^{14}C-residues and 84%-85% of the high dose ^{14}C-residues were excreted by the treated hens.

Table 7. Total radioactive residues in eggs and tissues from laying hens dosed with [phenyl-^{14}C]- or [pyrrole-^{14}C]Pirate at 28x and 134x ppm for seven consecutive days.

Matrix	TRR (Pirate equivalents, ppm)			
	28x dose		134x dose	
	[phenyl-^{14}C]	[pyrrole-^{14}C]	[phenyl-^{14}C]	[pyrrole-^{14}C]
Egg, Day 1	<0.01	<0.01	<0.01	<0.01
Day 2	<0.01	0.01	0.10	0.04
Day 3	0.02	0.04	0.18	0.14
Day 4	0.04	0.05	0.25	0.24
Day 5	0.06	0.05	0.33	0.31
Day 6	0.06	0.08	0.33	0.33
Day 7	0.07	0.08	0.42	0.37
Liver	0.23	0.28	1.25	1.31
Kidney	0.09	0.05	0.52	0.37
Skin	0.06	0.08	0.44	0.35
Muscle	<0.01	<0.01	0.02	0.02

Extraction of Residues

The data from the high dose hen samples were comparable to the available data from the low dose group. Therefore, the data from the low dose samples are not presented in this report.

Eggs were sequentially extracted with MeOH and MeOH:HCl (49:1; v:v). The extracts were combined, concentrated, and analyzed by HPLC. Insoluble egg residues were then extracted with hexane, and the remaining solids were re-extracted with ACN. Muscle samples were extracted by the same procedure used for eggs, except that the residues extracted into MeOH and MeOH:HCl were redissolved in ACN for HPLC analysis, and the non-extractable residues were not further analyzed.

Liver and kidney samples were sequentially extracted with MeOH, MeOH:HCl (99:1; v:v for liver and 49:1; v:v for kidney), and 0.5% Triton X-100/MeOH. The extracts were combined, concentrated to dryness, and redissolved in MeOH. The MeOH extract yielded an oil, MeOH, and precipitate fraction. The oil and MeOH fractions were analyzed by HPLC and the precipitate was combined with the non-extractable fraction, radioassayed, and reserved for hydrolysis (described below).

Skin/fat samples were extracted sequentially with hexane and ACN. The extracts were combined, concentrated to an oil, and extracted with ACN. The combined ACN fraction was filtered, concentrated, and analyzed by HPLC.

Hydrolysis of Residues

Subsamples of non-extractable liver fractions were hydrolysed sequentially as follows: (i) incubated with protease at 37 C for 20 hours and the released ^{14}C-residues were partitioned into MeOH:ACN (1:1; v:v), (ii) incubated with 6 N HCl at 37 C for 20 hours and the released radioactive residues were partitioned into MeOH, and (iii) refluxed with 6 N HCl for 17 hours and the hydrolysed residues were partitioned into MeOH. Non-extractable kidney fractions were hydrolysed with protease and mild acid. The petitioner reported that identification of ^{14}C-residues in the MeOH and ACN extracts of the enzyme and acid hydrolysates are in progress.

Characterization of Residues

The MeOH, ACN, and oil fractions were analyzed by HPLC using a C-18 column (System I) on a system equipped with UV and radioactivity detectors. Eluates were collected and analyzed for radioactivity by LSS. The limit of detection was 0.01 ppm. The C-18 HPLC elution profile was used to calculate the percentage of metabolites present in each extract. Metabolites resolved by the C-18 column were reanalyzed by HPLC using an amino reversed-phase column with several solvent systems (System II and III). The ^{14}C-residues in the egg and tissue extracts resolved by HPLC were compared to the elution profile of non-labeled reference standards. The identities of selected metabolites were confirmed by LC/MS or GC/MS. Representative chromatograms and scans were submitted.

The distribution of ^{14}C-activity in eggs and tissue samples from laying hens dosed with [phenyl-^{14}C]- and [pyrrole-^{14}C]Pirate are presented in Table 8 and summaries of the components characterized/identified are presented in Tables 9 and 10.

Table 8. Distribution of TRR in eggs and tissues from laying hens dosed with [phenyl-^{14}C]pirate or [pyrrole-^{14}C]Pirate at 134x for seven consecutive days.

Substrate	Fraction	Pirate equivalents		Characterization/Identification
		% TRR	ppm [a]	
Egg [phenyl-^{14}C] (0.42 ppm)	MeOH and MeOH/HCl	86	0.36	Pirate (40% TRR, 0.17 ppm) and CL 303,268 (31% TRR, 0.13 ppm) were identified by HPLC. Metabolites M-6 (4% TRR, 0.02 ppm), M-7A (3% TRR, 0.01 ppm), and other Unknowns (collectively 7% TRR, 0.03 ppm) were resolved by HPLC [b].
	Hexane	2	<0.01	Not analyzed = N/A
	ACN	2	<0.01	N/A
	Non-extractable	11	0.05	N/A
Egg [pyrrole-^{14}C] (0.38 ppm)	MeOH/HCl	84	0.32	Pirate (42% TRR, 0.16 ppm) and CL 303,268 (28% TRR, 0.11 ppm) were identified by HPLC. Metabolites M-6 (4% TRR, 0.02 ppm), M-7A (4% TRR, 0.02 ppm), and other Unknowns (collectively 6% TRR, 0.02 ppm) were resolved by HPLC.
	Hexane	2	<0.01	N/A
	ACN	3	0.01	N/A
	Non-extractable	12	0.05	N/A
Liver [phenyl-^{14}C] (1.25 ppm)	MeOH	34	0.43	Pirate (3% TRR, 0.04 ppm) and CL 303,268 (17% TRR, 0.21 ppm) were identified by HPLC. Metabolites M-1 (3% TRR, 0.04 ppm), M-5 (2% TRR, 0.03 ppm), M-6 (2% TRR, 0.02 ppm), M-7 + M-7A (5% TRR, 0.06 ppm), and other Unknowns (collectively 5% TRR, 0.06 ppm) were resolved by HPLC [c].
	Non-extractable	66	0.83	Hydrolysed with enzyme and acid.
	Protease hydrolysate	4	0.05	N/A
	HCl hydrolysate	16	0.20	N/A
	HCl reflux	44	0.55	N/A
	Non-extractable	2	0.03	N/A
Liver [pyrrole-^{14}C] (1.31 ppm)	MeOH	24	0.31	Pirate (2% TRR, 0.03 ppm) and CL 303,268 (7% TRR, 0.09 ppm) were identified by HPLC. Metabolites M-1 (3% TRR, 0.04 ppm), M-5 (1% TRR, 0.01 ppm), M-6 (1% TRR, 0.02 ppm), M-7 + M-7A (5% TRR, 0.07 ppm), and other Unknowns (collectively 5% TRR, 0.06 ppm) were resolved by HPLC.
	Non-extractable	76	1.00	Hydrolysed with enzyme and acid.
	Protease hydrolysate	2	0.03	N/A
	HCl hydrolysate	14	0.18	N/A
	HCl reflux	47	0.62	N/A
	Non-extractable	13	0.17	N/A
Skin/fat [phenyl-^{14}C] 0.47 ppm	Hexane/ACN	93	0.44	Pirate (84% TRR, 0.39 ppm) and CL 303,268 (4% TRR, 0.02 ppm) were identified by HPLC. Metabolite M-7A (4% TRR, 0.02 ppm), and other Unknowns (collectively 2% TRR, <0.01 ppm) were resolved by HPLC.
	Non-extractable	7	0.03	N/A

(continued; footnotes follow)

Table 8 *(continued)*.

Substrate	Fraction	Pirate equivalents		Characterization/Identification
		% TRR	ppm [a]	
Skin/fat [pyrrole-^{14}C] 0.37 ppm	Hexane/ACN	94	0.35	Pirate (79% TRR, 0.29 ppm) and CL 303,268 (5% TRR, 0.02 ppm) were identified by HPLC. Metabolite M-7A (2% TRR, 0.01 ppm), and other Unknowns (collectively 8% TRR, 0.03 ppm) were resolved by HPLC.
	Non-extractable	6	0.02	N/A
Kidney [phenyl-^{14}C] (0.52 ppm)	MeOH	79	0.41	Pirate (10% TRR, 0.05 ppm), CL 303,268 (17% TRR, 0.09 ppm), CL 325,195 (5% TRR, 0.02 ppm), and CL 322,250 (4% TRR, 0.02 ppm) were identified by HPLC. Metabolites M-1 (6% TRR, 0.03 ppm), M-3 (2% TRR, 0.01 ppm), M-4 (2% TRR, 0.01 ppm), M-6 (6% TRR, 0.03 ppm), M-7 (4% TRR, 0.02 ppm), M-7A (4% TRR, 0.02 ppm), M-10 (10% TRR, 0.05 ppm), and other Unknowns (collectively 9% TRR, 0.05 ppm) were resolved by HPLC [d].
	Non-extractable	21	0.11	Hydrolysed with enzyme and acid.
	Protease hydrolysate	13	0.07	N/A
	HCl hydrolysate	<2	<0.01	N/A
	Non-extractable	8	0.04	N/A
Kidney [pyrrole-^{14}C] (0.37 ppm)	MeOH	77	0.28	Pirate (11% TRR, 0.04 ppm) and CL 303,268 (25% TRR, 0.09 ppm) were identified by HPLC. Metabolites M-1 (10% TRR, 0.04 ppm), M-6 (2% TRR, 0.01 ppm), M-7 (5% TRR, 0.02 ppm), M-7A (2% TRR, 0.01 ppm), M-10 (10% TRR, 0.04 ppm), and other Unknowns (collectively 7% TRR, 0.03 ppm) were resolved by HPLC.
	Non-extractable	23	0.09	Hydrolysed with enzyme and acid.
	Protease hydrolysate	14	0.05	N/A
	HCl hydrolysate	<3	<.01	N/A
	Non-extractable	8	0.03	N/A
Muscle [phenyl-^{14}C] 0.02 ppm	ACN	61	0.01	Pirate (25% TRR, 0.01 ppm) and CL 303,268 (5% TRR, <0.01 ppm) were identified by HPLC. Metabolites M-6 (4% TRR, <0.01 ppm), M-7A (23% TRR, <0.01 ppm), and other Unknowns (collectively 5% TRR, <0.01 ppm) were resolved by HPLC.
	Non-extractable	39	<0.01	N/A
Muscle [pyrrole-^{14}C] 0.02 ppm	ACN	68	0.01	Pirate (31% TRR, <0.01 ppm) and CL 303,268 (7% TRR, <0.01 ppm) were identified by HPLC. Metabolites M-6 (5% TRR, <0.01 ppm), M-7A (11% TRR, <0.01 ppm), and other Unknowns (collectively 14% TRR, <0.01 ppm) were resolved by HPLC.
	Non-extractable	32	<0.01	N/A

• TRR (ppm) of HPLC analyzed organic fractions were calculated by study reviewer.

[b] Petitioner reported that the other unknown metabolites consisted of several radioactive components, each accounting for <0.01 ppm.

[c] The identification of pirate, M-6, M-7A, and M-8 isolated from hen liver were confirmed by GC/MS.

[d] The identification of M-3 isolated from hen feces (also found in kidney) were confirmed by GC/MS as a mixture of AC 8508-78-BA (sulfate conjugate of CL 322,250) and AC 8508-78-BB.

Table 9. Characterization/identification of radioactive residues in laying hens dosed with [phenyl-^{14}C]Pirate at 134x for seven consecutive days.

Metabolite/Fractions	Egg		Liver		Kidney		Skin/Fat		Muscle	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	PPM
CL 303,630 (Pirate)	40	0.17	3	0.04	10	0.05	84	0.39	25	0.01
AC 8508-78-BA/BB (M-3) [a]	--	--	--	--	2	0.01	--	--	--	--
CL 325,195 (M-5)	--	--	2	0.03	5	0.02	--	--	--	--
CL 322,250 (M-5A)	--	--	--	--	4	0.02	--	--	--	--
AC 8508-50-C (M6)	4	0.02	2	0.02	6	0.03	--	--	4	<0.01
AC 8944-45 (M-7)	--		5 [b]	0.06	4	0.02	--	--	--	--
AC 8508-33-B1 (M-7A)	3	0.01	--	--	4	0.02	4	0.02	23	<0.01
CL 303,268 (M-8)	31	0.13	17	0.21	17	0.09	4	0.02	5	<0.01
Total Identified	78	0.33	29	0.36	52	0.26	92	0.43	57	<0.04
Unknown M-1	--	--	3	0.04	6	0.03	--	--	--	--
Unknown M-4	--	--	--	--	2	0.01	--	--	--	--
Unknown M-10	--	--	--	--	10	0.05	--	--	--	--
Unknowns, other	7	0.03	5	0.06	9	0.05	2	<0.01	5	<0.01
Hexane extract	2	<0.01	--	--	--	--	--	--	--	--
ACN extract	2	<0.01	--	--	--	--	--	--	--	--
Protease hydrolysate	--	--	4	0.05	13	0.07	--	--	--	--
HCL hydrolysate	--	--	16	0.20	<2	<0.01	--	--	--	--
HCL reflux	--	--	44	0.55	--	--	--	--	--	--
Total Characterized	**89**	**0.38**	**101**	**1.26**	**94**	**0.48**	**94**	**0.44**	**62**	**<0.05**
Non-extractable residues	11	0.05	2	0.03	8	0.04	7	0.03	39	<0.01

[a] Identified/confirmed as a mixture of AC 8508-78-BA (sulfate conjugate of CL 322,250) and AC 8508-78-BB.
[b] Metabolites M-7 and M-7A were reported as a composite.

Table 10. Characterization and identification of radioactive residues in laying hens dosed with [pyrrole-^{14}C]Pirate at 134x for seven consecutive days.

Metabolite/Fraction	Egg		Liver		Kidney		Skin/Fat		Muscle	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	PPM
CL 303,630 (Pirate)	42	0.16	2	0.03	11	0.04	79	0.29	31	<0.01
CL 325,195 (M-5)	--	--	1	0.01	--	--	--	--	--	--
AC 8508-50-C (M6)	4	0.02	1	0.02	2	0.01	--	--	5	<0.01
AC 8944-45 (M-7)	--	--	5 •	0.07	5	0.02	--	--	--	--
AC 8508-33-B1 (M-7A)	4	0.02	--	--	2	0.01	2	0.01	11	<0.01
CL 303,268 (M-8)	28	0.11	7	0.09	25	0.09	5	0.02	7	<0.01
Total Identified	78	0.31	16	0.22	45	0.17	86	0.32	54	<0.04
Unknown M-1	--	--	3	0.04	10	0.04	--	--	--	--
Unknown M-10	--	--	--	--	10	0.04	--	--	--	--
Unknowns, other	6	0.02	5	0.06	7	0.03	8	0.03	14	<0.01
Hexane extract	2	<0.01	--	--	--	--	--	--	--	--
ACN extract	3	0.01	--	--	--	--	--	--	--	--
Protease hydrolysate	--	--	2	0.03	14	0.05	--	--	--	--
HCL hydrolysate	--	--	14	0.18	<3	<0.01	--	--	--	--
HCL reflux	--	--	47	0.62	--	--	--	--	--	--
Total Characterized	**89**	**0.35**	**87**	**1.15**	**89**	**0.34**	**94**	**0.35**	**68**	**<0.05**
Non-extractable residues	12	0.05	13	0.17	8	0.03	6	0.02	32	<0.01

• Metabolites M-7 and M-7A were reported as a composite.

Summary of hen metabolism: Laying hens were orally dosed with [phenyl-^{14}C]- and [pyrrole-^{14}C]Pirate at *ca.* 28x and 134x the proposed maximum daily dietary burden. The distribution of TRR in eggs and tissues from both labeled samples were similar. In the high dose groups, the TRR in eggs increased from <0.01 to 0.42 ppm by day 7. Radioactive residues ranged from 0.02 ppm in muscle to 0.52 ppm in kidney and were highest in liver (1.25-1.31 ppm).

The data indicate that laying hens metabolize [phenyl-^{14}C]- and [pyrrole-^{14}C]Pirate in a similar manner yielding similar metabolites. The parent compound and eight metabolites were detected in eggs and tissues; two metabolites (AC 8508-78-BA/BB) were identified as a mixture. The total identified/characterized ^{14}C-residues accounted for 89% TRR (eggs), ≥87% TRR (liver), ≤89% TRR (kidney), 94% TRR (skin/fat), and ≥62% TRR (muscle). The petitioner identified the parent compound as the major metabolite from [phenyl-^{14}C]- and [pyrrole-^{14}C]Pirate samples in eggs (40-42% TRR, 0.16-0.17 ppm), skin/fat (79-84% TRR, 0.29-0.39 ppm), and muscle (25-31% TRR, <0.01-0.01 ppm). In the liver and kidney, CL 303,268 was the major metabolite, which amounted to 7-17% TRR (0.09-0.21 ppm) and 17-25% TRR (0.09 ppm), respectively. Three other metabolites (CL 325,195, AC 8508-50-C, and AC 8508-33-B1) common to [phenyl-^{14}C]- and [pyrrole-^{14}C]-labeled samples of Pirate and two additional metabolites (AC 8508-78-BA/BB and CL 322,250) derived from [phenyl-^{14}C]Pirate were also identified. The chemical nature of Pirate, AC 8508-78-BA/BB, AC 8508-50-C, AC 8508-33-B-1, and CL 303,268 were confirmed by LC/MS or GC/MS (see Attachment 2). Components of unknown nature occurring at 3-10% TRR (0.01-0.05 ppm) included M-1, M-4 and M-10 and other unknowns, occurring each at <0.01 ppm were also characterized. Non-extractable residues in eggs and tissues accounted for 2-39% of the TRR (<0.01-0.17 ppm).

Comparative metabolism: The metabolism of orally administered [phenyl-^{14}C]- and [pyrrole-^{14}C]Pirate in laying hens is similar to that of lactating goats. In both species, the main metabolic route for Pirate metabolism is N-dealkylation and ring hydroxylation.

Radiolabeled Validation of Analytical Methods: Samples from the goat and hen metabolism studies were not analyzed using the proposed enforcement method. Radiovalidation of the method used for data collection and enforcement purposes using samples from the metabolism studies remains outstanding.

Residue Analytical Methods

American Cyanamid (1993; MRID 42770237) submitted validation data for GC/ECD methods for determining residues of parent Pirate in/on cottonseeds (method M-2216) and in cottonseed processed commodities (method M-2274). The submission also includes an independent laboratory validation of method M-2216 on cottonseeds. Untreated control samples of cottonseed commodities were fortified with Pirate at 0.05-0.50 ppm in the method validation studies.

For method M-2216, residues are extracted from cottonseeds with MeOH:water (85:15, v:v), filtered, and purified using a C-18 solid-phase extraction (SPE) column eluted with hexane. Residues are then evaporated to dryness and redissolved in hexane, and parent Pirate is quantified by GC/ECD. The validated limit of detection is 0.05 ppm. Samples from the validation study of method M-2216 were analyzed by American Cyanamid (Princeton, NJ) and the independent laboratory method validation was conducted by ABC Laboratories, Inc. (Columbia, MO).

Method M-2274 is a modification of method M-2216 utilizing different solvent systems depending on the matrix being analyzed. Residues in meal and hulls are extracted with MeOH:water (85:15, v:v) and cleaned using a C-18 SPE column washed with MeOH:water (1:2, v:v) and eluted with

hexane. Purified residues are then concentrated, redissolved in hexane, and quantified by GC/ECD. Residues in crude and refined oils are extracted with MeOH:ACN (50:50, v:v) and partitioned with heptane. Residues in the MeOH:ACN fraction are concentrated, redissolved in CH_2CL_2, and cleaned-up using a C-18 SPE column eluted with CH_2Cl_2. Residues are subsequently purified using a Florisil column eluted with acetone:hexane (5:95, v:v). Residues are then concentrated, redissolved in hexane, and analyzed by GC/ECD. Residues in soapstock are extracted with MeOH:ACN (5:95, v:v), and the residues are purified and analyzed using the same procedures described for crude and refined oil samples. The validated limit of detection is 0.05 ppm for all processed matrices. The petitioner provided representative chromatograms and sample calculations.

The recovery of Pirate from cottonseed commodities using GC/ECD methods M-2216 and M-2274 are presented in Table 11. Apparent residues of Pirate were nondetectable (<0.05 ppm) in/on five untreated control samples of cottonseed and in two untreated control samples of each processed commodity.

Table 11. Method recoveries of Pirate from fortified samples of cottonseed and its processed commodities.

Commodity	Fortification level (ppm)	Analytical Lab •	Number of Samples	Percent recovery
Method M-2216				
Cottonseed	0.05	ACC	3	88.0-94.0
		ABCL	2	81.6, 100
	0.10	ACC	3	88.0-92.0
		ABCL	2	96.0, 105
	0.50	ACC	3	84.0-85.0
		ABCL	4	89.4-97.6
Method M-2274				
Meal	0.05	ACC	2	91, 96
	0.50	ACC	2	84, 93
Hulls	0.05	ACC	2	98, 102
	0.50	ACC	2	77, 86
Crude oil	0.05	ACC	2	87, 97
	0.50	ACC	2	90, 95
Refined oil	0.05	ACC	2	83, 83
	0.50	ACC	2	80, 84
Soapstock	0.05	ACC	2	74, 91
	0.50	ACC	2	86, 90

• ACC: American Cyanamid Company; ABCL: Independent laboratory validation conducted by ABC Laboratories, Inc.

Samples of cottonseed commodities from the submitted storage stability (1993; MRID 42770239) and field residue studies (1993; MRID 42770238) were analyzed for residues of parent Pirate using methods M-2216 and M-2274. Table 12 shows concurrent method recoveries of Pirate from cottonseed commodities from the field residue and processing studies fortified with the parent compound at 0.05-2.53 ppm.

The available data indicated that methods M-2216 and M-2274 are adequate for determining residues of Pirate in/on cottonseed and in cottonseed processed commodities, respectively. These methods are adequate for enforcement of the tolerance expression (parent only) for this EUP and temporary tolerance petition. In addition, a successful independent laboratory validation was completed for method M-2216; however, the method should not be validated by EPA until the HED Metabolism Committee has decided what residues will be included in the tolerance expression. Prior to EPA lab validation, the petitioner must submit a reference standard of Pirate to the EPA repository, as well as the accompanying material safety data sheet (MSDS). Pirate should also be tested using FDA Multiresidue methods (PAM Vol. I).

Table 12. Concurrent method recoveries of Pirate from fortified samples of cottonseed and its processed commodities (1993; MRID 42770238).

Commodity	Fortification level (ppm)	Analytical Lab •	Number of Samples	Percent recovery
Method M-2216				
Cottonseed (1991)	0.05	ABCL	8	87.4-97.4
		ACC	3	84.0-96.0
	0.10	ABCL	2	96.1, 95.6
	0.50	ABCL	8	89.4-96.2
Cottonseed (1992)	0.05	ABCL	2	97, 98
		ACC	2	91, 98
	0.50	ABCL	2	99, 100
	2.53	ABCL	3	98-103
Method M-2274				
Meal	0.05	ACC	1	85
Hulls	0.05	ACC	1	95
Crude oil	0.05	ACC	1	83
Refined oil	0.05	ACC	1	90
Soapstock	0.05	ACC	1	102

• ABCL: ABC Laboratories, Inc.; ACC: American Cyanamid Company.

Storage Stability Data

American Cyanamid (1993; MRID 42770239) submitted interim data depicting the frozen storage stability of residues of Pirate in/on cottonseeds. The submitted data are from an interim report of an ongoing storage stability study that is to be 2 years in duration. Untreated control samples of cottonseed were fortified with Pirate at 0.25 ppm and stored frozen at an unspecified temperature. Samples were extracted and analyzed immediately after fortification and after 3 and 6 months of frozen storage. Apparent residues of Pirate were nondetectable (<0.05 ppm) in/on three control samples of cottonseeds. Samples were analyzed by American Cyanamid (Princeton, NJ) and ABC Laboratories (Columbia, MO) using GC/ECD method M-2216. The storage stability of Pirate in/on fortified cottonseed samples is presented in Table 13. The petitioner provided representative chromatograms and sample calculations. The submitted storage stability data indicate that residues of Pirate are stable under frozen storage conditions for up to 6 months in cottonseeds. No storage stability data for residues of Pirate in cottonseed processed commodities were submitted.

Table 13. Recovery of Pirate from cottonseeds fortified at 0.25 ppm and stored frozen.

Storage interval (months)	Percent Recovery [a]	Analytical Laboratory [b]	Concurrent Method Recovery (%)
0	98, 108	ACC	99
3	98, 98	ABCL	91
6	93, 99	ABCL	100

[a] Residue values are not corrected for concurrent method recoveries; each value represents one sample. [b] ABCL: ABC Laboratories, Inc.; ACC:American Cyanamid Company.

Cottonseed samples from the field residue study were stored at -20 C for 7.6-8.6 months (ACC) and 7.4-9.8 months (ABCL) prior to analysis; cottonseed samples from the processing study were stored frozen for 1.6-3.5 months prior to analysis (ABC); and samples of cottonseed processed commodities were stored at -20 C for 3.5-3.6 months (ACC).

For purposes of this EUP and temporary tolerance petition, the submitted storage stability data are adequate. The data indicate that residues of Pirate are stable under frozen storage conditions for up to 6 months in cottonseeds. No storage stability data for residues of Pirate in cottonseed processed commodities were submitted. Cottonseed samples from the field residue and processing studies were stored frozen for 1.6-9.8 months prior to analysis, and processed commodities were analyzed after approximately 4 months of frozen storage. For establishment of a permanent tolerance, additional storage stability data are required reflecting the maximum storage interval for cottonseeds and the stability of Pirate in processed matrices stored frozen for up to 4 months.

Magnitude of the Residue in Plants

Cottonseed

American Cyanamid submitted data (1993; MRID 42770238) from seven tests conducted in 1991 in AR(1), CA(1), LA(2), MS(2), and TX(1) and one test conducted in 1992 in LA depicting residues of Pirate in/on cottonseeds following the last of five foliar broadcast applications of the 2 and 3 lb/gal EC formulations (EPA file No. 241-EUP) each at 0.4 lb ai/A/application. The total seasonal application rate was 2 lb ai/A (1x the maximum proposed seasonal application rate). Applications were made at 7-day intervals using back pack or tractor mounted spray equipment at 10-20 gal/A, with the last application being made when 5 to 70% of the bolls were opened. Cottonseeds from the 1991 trials were harvested from 21-39 days after the final application and cottonseeds from the 1992 trial were harvested from 0-28 days after the final application. Cottonseeds were ginned after harvest and stored frozen at -24 to -18 C. Samples analyzed at ACC were stored frozen at -20 C for 7.6-8.6 months prior to analysis. Samples from the 1991 and 1992 trials analyzed by ABCL were shipped overnight and stored frozen for 7.4-9.8 and 1.6-3.5 months, respectively, prior to analysis.

Cottonseeds were analyzed for residues of parent Pirate using GC/ECD method M-2216, which has a detection limit of 0.05 ppm. Apparent residues of Pirate in/on 26 control samples of cottonseeds were nondetectable (<0.05 ppm). Concurrent method recoveries from 30 control samples fortified with Pirate at 0.05-2.53 were 84-103%. Uncorrected residues of Pirate in/on treated cottonseeds are presented in Table 14.

Table 14. Residues of Pirate in/on cottonseeds following the last of five foliar broadcast applications, made at 7-day intervals, of the 2 or 3 lb/gal EC formulations at 2 lb ai/A (1x).

Trial Year	PTI [a] (Days)	Test States	Number of Samples	Residues (ppm)
1991	**21 [b]**	**AR, CA, LA, MS, TX**	**6**	**<0.05-0.26**
	24	LA	1	0.13
	28	AR, CA, LA, MS	6	<0.05-0.24
	30	TX	1	<0.05
	35	CA, LA, MS	3	<0.05-0.15
	36	LA, TX	2	<0.05, 0.17
	38	MS	1	0.12
	39	AR	1	0.16
1992	0	LA	1	1.51
	7	LA	1	0.61
	14	LA	1	0.80
	21	**LA**	**1**	**0.32**
	28	LA	1	0.31

[a] Posttreatment interval.
[b] AR, CA, LA, MS, TX Pirate residues were 0.09, 0.26, 0.23, <0.05 to 0.24, and <0.05 ppm, respectively at a 21 day PHI. Data representing the proposed PHI of 21 days are bolded.

Geographic representation is adequate. The tests States of AR(7%), CA(18%), LA(7%), MS(12%), and TX(33%) accounted for 77% of the 1990 U.S. cottonseed production (Agricultural Statistics 1991, p. 106). Residues of Pirate were ≤0.32 ppm in/on seven cottonseed samples harvested 21 days following the last of five foliar broadcast applications of the 2 and 3 lb/gal EC formulations at 1x the proposed maximum seasonal application rate.

For purposes of this EUP and temporary tolerance petition, the available data indicate that residues of parent Pirate are not likely to exceed the proposed temporary tolerance of 0.5 ppm in/on cottonseeds harvested 21 days following the last of 5 foliar broadcast applications of Pirate each at 0.4 lb ai/A, for a seasonal maximum rate of 2 lb ai/A. For a permanent tolerance additional field trial data representing commercial application procedures will be needed. A minimum of an additional 5 field trials in geographically representative areas (i.e. with two composite samples recommended per site) are required with at least one field trial conducted in either Georgia, North Carolina or South Carolina.

Cottonseed Processed Commodities

American Cyanamid submitted data (1993; MRID 42770238) depicting the concentration of residues of Pirate in cottonseed processed commodities. In a test conducted in LA in the 1992 season, cottonseeds were harvested 14 days following the last of five foliar applications of the 3 lb/gal EC formulation at 0.4 lb ai/A/application (1x). Applications were made at 7-day intervals using ground equipment at 16.5-18.8 gal/A. The last application was made when 45-55% of bolls were opened.

Treated and control samples were harvested, stored frozen at -24 to -19 C, and shipped in a freezer truck to the Engineering Biosciences Research Center of Texas A&M University (Bryan, TX) for processing. Cottonseeds were processed into hulls, meal, crude oil, refined oil, and soapstock using a simulated industrial procedure. In brief, the lint cotton was saw ginned to remove a majority of the lint and the seed was saw delinted to remove a majority of the existing lint. The delinted seed was mechanically cracked and screened to separate the majority of the hull material from the kernal material. The kernal material with some hull material was heated, flaked, expanded into collets, and exposed to hexane to remove the crude oil from the collet. The spent collets were desolventized with warm forced air. After the crude oil and hexane mixture was adjusted to the proper ratio, the crude oil was miscella refined.

The processed fractions were then packed in dry ice and shipped to the analytical laboratory (American Cyanamid, Princeton, NJ). Untreated control and treated samples were analyzed for residues of Pirate using Method M-2274, which has a detection limit of 0.05 ppm. Concurrent method recoveries were 85-102% from six control samples (one sample per commodity) fortified at 0.05 ppm. Apparent residues of Pirate were nondetectable (<0.05 ppm) in each untreated control sample. Uncorrected residues in treated cottonseed were 0.66 ppm. Uncorrected residues were 0.36 ppm in hulls, 0.1 ppm in refined and crude oils, and <0.05 ppm in meal and soapstock.

Although, the processing study indicates that residues of the parent Pirate do not concentrate in cottonseed processed commodities several study questions need to be resolved. The submitted processing data are only marginally adequate for the subject EUP only, because of a discrepancy in the mass balance for Pirate. Also, there were some problems in the study including loss of crude oil due to operator error and the lack of detailed data on the actual physical parameters (e.g. temperature, time, equipment) used in the processing study. Accordingly, no feed/food additive tolerances will be required for cottonseed processed commodities for the subject EUP only.

Additional information on the processing study are required including residue levels in the delinted cottonseed used for processing as well as residues in the lint and trash, copies of the sponser processing protocol and the exact protocol used including all deviations made and the complete processing report (i.e. including the temperature and duration of solvent extraction and equipment used), original processing data and calculations as well as submission of data depicting the frozen storage stability of residues of Pirate in cottonseed processed commodities compared to sample storage intervals used in the processing study. An explanation of the discrepancy in the mass balance needs to be resolved (i.e. significantly higher Pirate residues in the RAC than in any of the processed fractions). A new mass balance providing the disposition of 90 to 100% of the Pirate in the cottonseed used for processing is required. If Pirate instability is claimed to be a major reason for the discrepancy in the mass balance, data on the stability of Pirate in hexane at solvent extraction temperatures and durations representative of commercial processing procedures and resulting metabolites will be needed. A new cottonseed processing study representative of commercial procedures will likely be required for a permanent tolerance unless these issues are adequately resolved.

Magnitude of the Residue in Meat, Milk, Poultry, and Eggs

No feeding studies were submitted with this petition. Based on a worst case diet consisting of 25% cottonseeds, 15% cottonseed meal, and 15% cottonseed hulls (each at the 0.5 ppm proposed tolerance for cottonseed) and 40% alfalfa forage (at 0 ppm), the maximum daily dietary intake of Pirate by ruminants is estimated to be (.25 + .15 + .15 x 0.5 = .275/89 % dry matter) or 0.31 ppm. Based on a worst case poultry diet consisting of 20% cottonseed meal (at 0.5 ppm proposed tolerance) and 80% grain (at 0 ppm), the maximum daily dietary intake of Pirate by poultry is estimated to be (.20 x 0.5 = .10/89% dry matter) or 0.11 ppm.

For the purposes of this EUP and temporary tolerance petition only, CBTS concludes that residues of Pirate are not likely to be found in animal commodities, based on the feeding levels of the seven day metabolism studies (i.e. 3 to 17.9 ppm for ruminants or up to 58X and 3 to 15 ppm for poultry or up to 136X) and the resulting TRR. For the establishment of a permanent tolerance, a poultry feeding study is not likely to be required based on the total C14 residues in the metabolism study. However, a ruminant feeding study may be required (e.g. a common moiety method might result in detectable residues in the liver; total C14 residue in liver or 1.45 ppm / 58x = .025 ppm). A final decision on the need for poultry or ruminant feeding studies is deferred pending resolution of deficiencies relating to animal metabolism and a subsequent review by the HED Metabolism Committee

Other Considerations

An International Residue Limit Status sheet is included in this review as Attachment 3. Since no Codex, Canadian, or Mexican limits/tolerances have been established for Pirate, there are no compatibility problems at this time.

Attachment 2 - Proposed Metabolic Pathway for Pirate in Ruminants and Poultry
Attachment 3 - International Residue Limit Status Sheet
Attachment 4 - Pirate Product Chemistry Review Dated 10/22/93.
Attachment 5 - Confidential Appendix - Pirate Product Chemistry

MASTER RECORD IDENTIFICATION NUMBER

Citations for the MRID documents referred to in this review are presented below.

42770234 Mallipudi, N.M. (1993) CL 303,630: Metabolism of Carbon-14 Labeled CL 303,630 in Cotton under Field Conditions. Laboratory Project Study No. MET 93-016. Unpublished study conducted by Pan-Agricultural Laboratories, Inc. (Madera, CA) and American Cyanamid Co. (Princeton, NJ). 165 p.

42770235 Zulalian, J. (1993) CL 303,630: Metabolic Fate of Carbon-14 Labeled CL 303,630 in the Tissues and Milk of a Lactating Goat. Study No. MET 93-007; Unpublished study conducted and submitted by American Cyanamid Company, Princeton, NJ. 267 p.

42770236 Kao, L. M. (1993) CL 303,630: Metabolic Fate of [^{14}C] CL 303,630 in Laying Hens (Interim Report). Study No. MET 93-020; Unpublished study conducted and submitted by American Cyanamid Company, Princeton, NJ. 195 p.

42770237 Kim, D. (1993) CL 303,630: Validation of Methods of the Determination of CL 303,630 Residues in Cottonseed and Cottonseed Processing Commodities including Independent Validation. Laboratory Project Study Nos. RES 93-016, -078, and C3911. Unpublished study performed by American Cyanamid Company (Princeton, NJ) and ABC Laboratories, Inc. (Columbia, MO) and submitted by American Cyanamid Company (Princeton, NJ). 108 p.

42770238 Schaefer, T. (1993) CL 303,630: Residues of CL 303,630 in Ginned Cottonseed and in Processed Cottonseed Commodities (1991, LA-1992). Laboratory Project Study Nos. RES 93-003, -004, -006, -007, -035, -036, -037, and -080. Unpublished study performed and submitted by American Cyanamid Company (Princeton, NJ). 603 p.

42770239 Kim, D. (1993) Interim Report: Freezer Stability Study for PIRATE Insecticide-Miticide in Cottonseed. Laboratory Project Protocol No. XD92PT06. Unpublished study performed and submitted by American Cyanamid Company (Princeton, NJ). 9 p.

12/5/92

INTERNATIONAL RESIDUE LIMIT STATUS

CHEMICAL PIRATE (NEW CHEMICAL)

CODEX NO. _____

CODEX STATUS:

☑ No Codex Proposal Step 6 or above

Residue(if Step 8): _____

Crop(s)	Limit (mg/kg)

PROPOSED U.S. TOLERANCES:

Petition No. _____

RCB Reviewer OTAKIE

Residue: 4-bromo-2-(chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile

Crop(s)	Limit (mg/kg)
COTTON SEED	0.5

CANADIAN LIMITS:

☑ No Canadian limit

Residue: _____

Crop(s)	Limit (mg/kg)

MEXICAN LIMITS:

☑ No Mexican limit

Residue: _____

Crop(s)	Limit (mg/kg)

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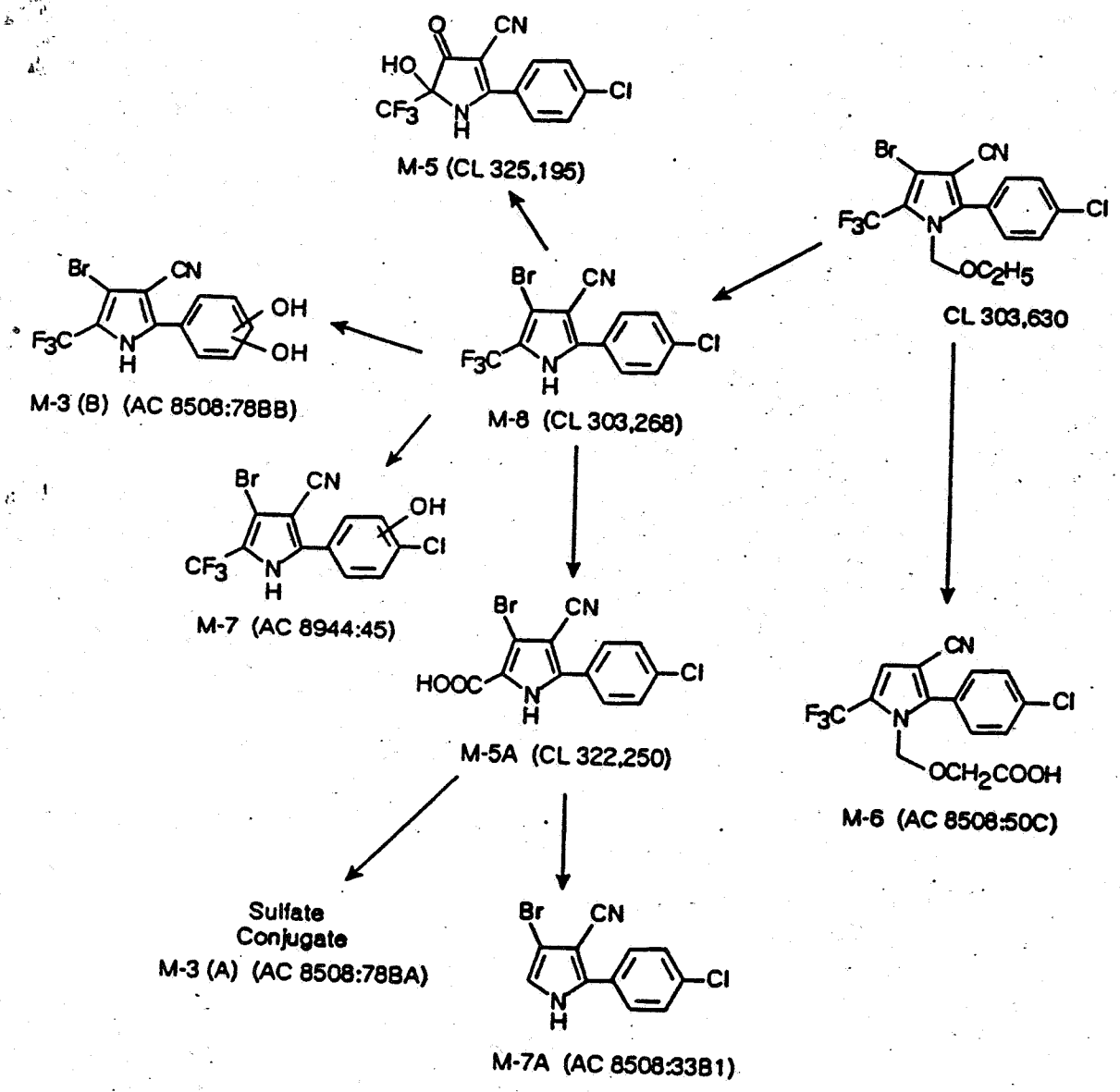
NOTES:

Form revised 1986

ATTACHMENT 2

The Proposed Metabolic Pathway of CL 303,630 in Laying hens AND RUMINANTS



DYNAMAC
CORPORATION
Environmental Services

Final Report

PIRATE
(CBTS)

TASK 4
Product Chemistry Data Submitted in Support of an Experimental Use Permit and Temporary Tolerance for Cottonseed

October 22, 1993

Contract No. 68-D2-0053

Submitted to:
U.S. Environmental Protection Agency
Arlington, VA 22202

Submitted by:
Dynamac Corporation
The Dynamac Building
2275 Research Boulevard
Rockville, MD 20850-3268

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PIRATE

Task 4: CBTS

PRODUCT CHEMISTRY DATA SUBMITTED IN SUPPORT OF AN EXPERIMENTAL USE PERMIT

BACKGROUND

American Cyanamid Company is currently seeking an experimental use permit for the insecticide-miticide Pirate™ 34.3% end-use product (EP; no EPA file symbol assigned) on cotton. In support of the experimental use permit, American Cyanamid has submitted three volumes of product chemistry data (1993; MRIDs 42770201 through 42770203) for a Pirate 94.5% technical (T; no EPA file symbol assigned). Data submitted concerning the 34.3% EP (1993; MRIDs 42770204 through 42770206) are not reviewed in this document.

INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act [FIFRA §3(c)(2)(A)] requires the Environmental Protection Agency to establish guidelines for registering pesticides in the United States. The Agency, in turn, requires registrants to provide quantitative data on all added ingredients, active and inert, which are equal to or greater than 0.1% of the product by weight.

To establish the composition of products to be registered, the Agency requires detailed information on the manufacturing process and/or formulation processes, and a discussion of the formation of manufacturing impurities. Furthermore, to assure that the composition of the product as marketed will not vary from that evaluated at the time of registration, prospective pesticide registrants are required to propose certified upper and lower composition limits for the added ingredients, and upper limits for toxicologically significant impurities. Standard certified limits for pesticide product ingredients are established according to 40 CFR §158.175(b)(2); these limits may be modified with appropriate and acceptable explanation by the registrant.

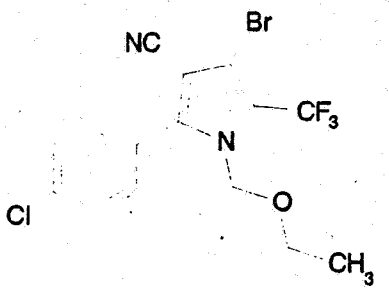
The Agency also requires data on the physical and chemical properties of the pesticide active ingredient and its formulations, such as melting and boiling points, ambient vapor pressures, and solubility in various solvents. Corresponding to each of the Topical Discussions listed below are the Guideline Reference Numbers from "Pesticide Assessment Guidelines - Subdivision D - Product Chemistry", referred to in Title 40 of the Code of Federal Regulations (40 CFR), Part 158, "Data Requirements for Registration", Subpart C, "Product Chemistry Data Requirements". These regulations and guidelines explain the minimum data that the Agency needs to adequately assess the product chemistry of Pirate.

	Guidelines Reference No. from 40 CFR §158.155-190
Product Composition and Manufacture	61-(1-3)
Analysis and Certification of Product Ingredients	62-(1-3)
Physical and Chemical Characteristics	63-(2-20)

PRODUCT IDENTITY AND COMPOSITION

61-1. Product Identity and Disclosure of Ingredients

The active ingredient (ai) in the 94.5% T produced by American Cyanamid is 4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile. The chemical structure is:



Other identifying characteristics and codes are:

Empirical Formula:	$C_{15}H_{11}BrClF_3N_2O$
Molecular Weight:	407.6
CAS Registry No.:	122453-73-0

American Cyanamid has submitted (1993; MRID 42770201) product identity data including a Confidential Statement of Formula (CSF) dated 4/15/93 for the 94.5% T. Refer to the Confidential Appendix for disclosure of the ingredients in the technical product. The submitted data do not satisfy the requirements of 40 CFR §158.155 (Guideline Reference No. 61-1) regarding product identity for the American Cyanamid 94.5% T because the CSF lists impurities resulting from two different manufacturing processes (see GLN 61-3). The registrant must clarify which method is the current process proposed for production. If both methods are to be used, the registrant must determine if the two different processes produce basic and alternate formulations of the 94.5% T as defined under 40 CFR §152.43, and submit revised CSFs for the two formulations. We note that the impurities are listed on the CSF by their American Cyanamid chemical code; the commonly accepted chemical names are required on EPA Form 8570-4 (Rev. 12/90). The following additional data are required:

- A CSF must be submitted for the single, defined composition of the Pirate technical. Separate CSFs must be submitted for basic and alternate formulations (40 CFR §152.43). Components present at greater than or equal to 0.1% or of toxicological concern must be listed by their chemical names on EPA Form 8570-4 (Rev. 12/90).

61-2. Starting Materials and Manufacturing Process

American Cyanamid submitted (1993; MRID 42770201) the names and addresses of the suppliers of the starting materials and a description of the manufacturing process for the 94.5% T. Also included in the submission were the chemical equations for each intended reaction of each step of the process, the stoichiometric amounts of the starting materials, the order in which the starting materials are added, and a description of the conditions controlled during each step of the process. The submitted data are presented in the Confidential Appendix. These data do not satisfy the requirements of 40 CFR §158.160-162 (Guideline Reference No. 61-2) regarding the starting materials and manufacturing process of the American Cyanamid 94.5% T because two methods

(Method A and Method B) were submitted for the final step of the manufacture of the Pirate technical. Under discussion of formation of impurities, the registrant indicates that Method A and Method B result in different impurities. The registrant must clarify which method is the current process proposed for production. If both methods are to be used, the registrant must determine if the different processes produce basic and alternate formulations of the 94.5% T as defined under 40 CFR §152.43. In addition, the registrant discussed alternative methods for Method B under GLN 61-3 which need to be detailed, and the duration of each step and of the entire process must be submitted.

[redacted]

A description of the beginning materials and the manufacturing process for small-scale batch production of Pirate tebuconazole is included in the Confidential Appendix.

The following additional information is required for a permanent tolerance. CB concludes the following information, representing the full-scale (i.e., commercial) production process, is required to achieve full registration:

Manufacturing Process (i.e., on the full-scale production process)

a. Statements of whether the steps in the process are batch and/or continuous.

b. The amounts (e.g., weight) of the beginning materials and the order in which they are added.

c. A flowchart with chemical equations of each intended chemical reaction occurring at each step of the process, together with a complete description of the equipment used to produce and purify the product (e.g., reaction vessels, mixers, distillation and purification equipment, etc.).

d. If both manufacturing processes are to be used, the registrant must determine if Method A and Method B produce basic and alternate formulations as defined under 40 CFR §152.43.

e. A complete description of the physical conditions and control parameters (e.g., temperature, pressure, humidity, mixer RPM, etc.) must be provided for each step of the process, together with a discussion of the acceptable parameter range and influence on the purity and the relative amounts and/or identity of impurities, variation of these control parameters can cause.

f. A statement of the intended chemical reactions (if any) together with a flow chart with the chemical equations for each chemical reaction occurring at each step of the process.

g. The approximate time (e.g., duration) of each step in the production process.

h. A discussion of the measures taken to assure the quality of the final product.

Note: The required information on the beginning materials and manufacturing process may be deferred until after full-scale production commences, in accordance with an approved schedule for submission of the data (see GRN 62-1).

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61-3. Discussion of the Formation of Impurities

American Cyanamid submitted (1993; MRID 42770201) a discussion of the formation of impurities in the 94.5% T. The discussion included numerous confirmed and theoretical impurities formed as a result of carryover of the starting materials or their impurities, and intended and side reactions occurring during the manufacture of the 94.5% T including Methods A and B. The registrant also detailed product quality assurance and stated that no impurities are formed as a result of post-production degradation or carryover from equipment used to produce other products. These data satisfy the requirements of 40 CFR §158.167 (Guideline Reference No. 61-3) regarding discussion of formation of impurities in the American Cyanamid 94.5% T for the proposed temporary tolerance only.

Data has been submitted by the registrant on the small-scale production process, in response to this requirement. To achieve full registration, the petitioner must discuss the following information based on the full-scale production process for the technical or propose an acceptable schedule for submission of these data (see GRN 62-1):

1. For each impurity which may be present in the product at a level equal to or greater than 0.1 percent (1000 ppm) based on knowledge of:

   a. The composition of each beginning material and intentionally added inert ingredient;

   b. Impurities which are known to be present from other information;

   c. The substances which result from the intended reactions of the manufacturing process;

   d. Degradation or postproduction reactions of any of the product's ingredients;

   e. Contamination of the product from earlier use of the same production equipment to produce other substances or contamination from packaging materials; and

   f. Process control, purification, and quality control procedures used.

2. Any other impurity which was found to be present in any analysis of the product.

ANALYSIS AND CERTIFICATION OF PRODUCT INGREDIENTS

62-1. Preliminary Analysis

American Cyanamid submitted data (1993; MRID 42770202) from the preliminary analysis of five samples of the 94.5% T. Three batches were laboratory-scale productions representative of the manufacturing process, while the remaining two samples were 50-gallon preparations for TOX studies manufactured by an "earlier process". These data are presented in the Confidential Appendix. The submission does not satisfy the requirements of 40 CFR §158.170 (Guideline Reference No. 62-1) regarding preliminary analysis for the American Cyanamid 94.5% T because the preliminary analysis study must be submitted on five representative batches of the final product manufactured by the full scale production process (i.e. in commercial quantities). The registrant must also identify which of the manufacturing process discussed under GLN 61-2 the samples

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support. Preliminary analysis samples must be representative of the process to be used for production. Finally, the total closure of the analysis was approximately 98% which includes 0.54% of "other GC components". This leaves more than 2% of the technical unidentified. The following additional data are required:

To achieve full registration, the applicant must provide the following information:

- Analysis of five samples representing five different production runs of the final full scale production process for the active ingredient and each impurity. Data on the size of each production run (i.e., pounds or gallons of product produced) must also be provided. If the product is produced by a batch process, each sample should be taken from a different batch of the product and if the product is produced by a continuous process, samples should be taken at intervals sufficiently spaced to provide data on any variation in product content. All components present at greater than or equal to 0.1% must be identified and quantitated, and the analysis must demonstrate good total closure.

Alternatively, if the applicant considers it impractical to construct facilities to produce the proposed product in commercial quantities prior to receiving full registration so that the required preliminary product analyses on the full-scale production process can be provided, a deferral request must be submitted in accordance with the PAG Subdivision D -Product Chemistry, October 1982 (see pages 42, 43, 49, 50, and 51), with the proposal of an acceptable schedule for submission of these data.

62-2. Certified Limits

American Cyanamid submitted data (1993; MRID 42770202) which established certified limits for the 94.5% T. Data are presented in the Confidential Appendix, and do not satisfy the requirements of 40 CFR §158.175 (Guideline Reference No. 62-2) regarding certified limits for the American Cyanamid 94.5% T because the CSF lists impurities resulting from two different manufacturing processes (see GLN 61-3). The registrant must clarify which method is the current process proposed for production. If both methods are to be used, the registrant must determine if the different processes produce basic and alternate formulations of the 94.5% T as defined under 40 CFR §152.43, and submit revised CSFs for the two formulations. In addition, the proposed certified limits of the ai exceed the recommended range (±3%; 40 CFR §158.175(b)(2)). Although the registrant has included an explanation, the preliminary analysis data support narr ower certified limits.

Certified limits of the active ingredients and the known impurities present at levels ≥ 0.1 percent in the technical from the small-scale batch production process have been are listed in the CSF dated April 15, 1993. Based on the current manufacturing process the potential for formation of polyhalogenated dibenzo-p-dioxins and/or dibenzofurans is negligible (see 1/10/94 note of S. Funk) and N-nitrosamines at concentrations greater than 1 ppm are not expected, since there are no nitrosation steps or nitrated products. To achieve full registration, data on the preliminary analysis (see GRN 62-1) of the technical from the full-scale production with proposed certified limits reflecting these data will be required. The following additional data are required for a permanent tolerance:

- A CSF must be submitted for the single, defined composition of the Pirate technical and separate CSFs must be submitted for basic and alternate formulations (40 CFR §152.43), with all certified limits representative of the required preliminary analysis data (i.e. five batch analyses from the full scale production process) for each different manufacturing process. In addition, the registrant must propose more appropriate certified limits for the active ingredient or submit a justification for the proposed limits.

62-3. Enforcement Analytical Methods

American Cyanamid submitted (1993; MRID 42770202) a high-resolution gas chromatography (HRGC) method (M-2006.1) for the determination of Pirate per se in the 94.5% T. Samples and standards are prepared with the internal standard solution, ethyl stearate in chloroform, and diluted with acetonitrile. These solutions are then injected onto an isothermal capillary column (30 m x 0.25 mm ID, Durabond DB-1, 0.25-μm film thickness). The GC system is operated at 220 C and is equipped with a flame ionization detector (FID). A standard curve is generated for a concentration range of 0.5-3 mg/mL Pirate. The Pirate analyte is quantitated against the Pirate standard using ethyl stearate as an internal standard. Method accuracy was validated using linearity as the criterion (coefficient = 0.99999). Precision was validated for variance, dependent on analyst, days, weighings, and injections; the overall RSD was 0.401%. In addition, data pertaining to method specificity and ruggedness were submitted. The submitted validation data support the analytical method used for the determination of the active ingredient.

American Cyanamid has also submitted (1993; MRID 42770202) enforcement analytical methods for the determination of the impurities of the 94.5% T. These analytical methods are presented in the Confidential Appendix.

The submitted methods satisfy the requirements of 40 CFR §158.180 (Guideline Reference No. 62-3) regarding enforcement analytical methods for the 94.5% T. No additional data are required for the proposed temporary tolerance. However, any additional enforcement analytical methods required based on the products final composition resulting from full scale production will be required for a permanent tolerance.

PHYSICAL AND CHEMICAL CHARACTERISTICS

American Cyanamid submitted data (1993; MRID 42770203) regarding the physical and chemical properties of Pirate, including a complete description of the methods used. The submitted data are presented in Table 1 and satisfy the requirements of 40 CFR §158.190 (Guideline Reference No. 63-2 through 63-20) for the American Cyanamid Pirate 94.5% T except that data are required reflecting the stability of the TGAI on exposure to sunlight and to metals and metal ions. Additional data are required.

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Table 1. Physical and chemical properties of the Pirate manufacturing-use product (MP), technical grade of the active ingredient (TGAI) and the purified active ingredient (PAI). Data are from MRID 42770203 (1993).

Guidelines Reference No., 40 CFR §158.190; Name of Property		Description [Method] (test substance)
63-2.	Color	Munsell 10YR (9/1); light tan or light yellow [ASTM D1535-80] (TGAI/MP)
63-3.	Physical state	powdered solid [Pesticide Assessment, Subdivision D] (TGAI/MP)
63-4.	Odor	characteristic of halides and ketones [ASTM D1292-86] (TGAI/MP)
63-5.	Melting point	100-101 C [melting point apparatus] (TGAI)
63-6.	Boiling point	N/A; TGAI is a solid
63-7.	Density, bulk density, or specific gravity	0.543 g/mL tapped bulk density 0.355 g/mL untapped bulk density [CIPAC MT 33] (TGAI/MP)
63-8.	Solubility	Solvent — Solubility at 25 C deionized water — 0.12 mg/L water, pH 4 — 0.13 mg/L water, pH 7 — 0.14 mg/L water, pH 10 — 0.12 mg/L hexane — 0.89 g/100 mL methanol — 7.09 g/100 mL acetonitrile — 68.4 g/100 mL toluene — 75.4 g/100 mL acetone — 114 g/100 mL dichloromethane — 141 g/100 mL [HPLC assay] (TGAI)
63-9.	Vapor pressure	$< 1.0 \times 10^{-7}$ mm Hg at 25 C [gas saturation] (PAI; 98.8%)
63-10.	Dissociation constant	since there are no ionizable groups in the Pirate structure, no dissociation will occur (PAI)
63-11.	Octanol/water partition coefficient	K_{ow} = 67,670 (log K_{ow} = 4.83) at 25 C [EPA-600/4-79-032] (PAI; 97.7%)
63-12.	pH	7.16; 1% aqueous suspension at 24 C [OTS CG-1450, ASTM E 70-74] (TGAI/MP)
63-13.	Stability	stable at 25 C for 18 months, 37 C for 12 months, and 45 C for 3 months; the registrant claims data are not required concerning the stability of the TGAI to metals, metal ions or sunlight as the TGAI does not normally come in contact with any of these [GC assay] (TGAI)

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Guidelines Reference No., 40 CFR §158.190; Name of Property		Description [Method] (test substance)
63-14.	Oxidizing or reducing	unreactive to oxidizing or reducing agents; no reaction was observed when exposed to tap water, 1% monoammonium phosphate, 0.01M aqueous potassium permanganate and zinc foil (MP)
63-15.	Flammability	N/A; TGAI is a solid
63-16.	Explodability	not sensitive to an impact of 2 kg/cm at room temperature; one exotherm at 183 C with a heat release of -350 kJ/kg in differential thermal analysis; dust did not ignite at any concentration or ignition delay time tested; classified as Class 0 dust [impact, differential thermal analysis, and dust explositivity assays] (MP)
63-17.	Storage Stability	stable at warehouse conditions for 12 months [GC and HPLC assays] (MP)
63-18.	Viscosity	N/A; TGAI is a solid
63-19.	Miscibility	N/A; TGAI is a solid
63-20.	Corrosion Characteristics	no corrosion observed after 12 months storage in a polyethylene bag or a VELOSTAT® (non-conductive plastic) bag inside a fiberpak (MP)

MASTER RECORD IDENTIFICATION NUMBERS

References (used):

42770201 Doehner, R.F. (1993) Product Identity, Description of Beginning Materials and Manufacturing Process, and Theoretical Discussion of Impurities for PIRATE® Insecticide-Miticide Technical, Lab Project Numbers CHDV-33-6, CHDV-33-7. Unpublished study prepared by American Cyanamid Company. 85 p.

42770202 Fotiou, F. and Kirzecky, N.D. (1993) Preliminary Analysis, Certification of Limits, and Analytical Methods for PIRATE® Insecticide-Miticide Technical, Lab Project Numbers APBR-246 through APBR-249. Unpublished study prepared by American Cyanamid Company. 182 p.

42770203 Rahaman, Reza S., et. al. (1993) Physical and Chemical Characteristics of PIRATE® Insecticide-Miticide Technical, Lab Project Numbers HWI 6123-189, E-91-24, E-90-32, E-90-30, E-90-28, P 67, P 66. Unpublished study prepared by American Cyanamid Company. 228 p.

References (not used):

[The following MRIDs contain data pertaining to end-use products.]

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42770204 Schaaf, M. (1993) Product Identity, Description of Beginning Materials and Manufacturing Process, and Theoretical Discussion of Impurities for PIRATE® Insecticide-Miticide End-Use Formulation. Unpublished study prepared by American Cyanamid Company. 14 p.

42770205 Banick, W.M. and Schaaf, M. (1993) Certification of Limits and Analytical Method to Verify Certified Limits of PIRATE® Insecticide-Miticide End-Use Formulation, Lab Project Number C-4101. Unpublished study prepared by American Cyanamid Company. 51 p.

42770206 Schaaf, M. (1993) Physical and Chemical Characteristics for PIRATE® Insecticide-Miticide End-Use Formulation, Lab Project Numbers F-1145, F-1176. Unpublished study prepared by American Cyanamid Company. 50 p.

Chemical Name: Pirate
Registrant: American Cyanamid Company, Inc.
Product(s): 94.5% T (no EPA file symbol assigned)

PRODUCT CHEMISTRY DATA SUMMARY

Guideline Number	Requirement	Requirement Fulfilled? [a]	MRID Number
61-1	Product Identity and Disclosure of Ingredients	N [b]	42770201
61-2	Starting Materials and Manufacturing Process	N [c]	42770201
61-3	Discussion of Formation of Impurities	Y	42770201
62-1	Preliminary Analysis	N [d]	42770202
62-2	Certification of Ingredient Limits	N [b]	42770201, 42770202
62-3	Analytical Methods to Verify the Certified Limits	Y	42770202
63-2	Color	Y	42770203
63-3	Physical State	Y	42770203
63-4	Odor	Y	42770203
63-5	Melting Point	Y	42770203
63-6	Boiling Point	N/A	
63-7	Density, Bulk Density or Specific Gravity	Y	42770203
63-8	Solubility	Y	42770203
63-9	Vapor Pressure	Y	42770203
63-10	Dissociation Constant	Y	42770203
63-11	Octanol/Water Partition Coefficient	Y	42770203
63-12	pH	Y	42770203
63-13	Stability	N [e]	42770203
63-14	Oxidizing or Reducing Action	Y	42770203
63-15	Flammability	N/A	
63-16	Explodability	Y	42770203
63-17	Storage Stability	Y	42770203
63-18	Viscosity	N/A	
63-19	Miscibility	N/A	
63-20	Corrosion Characteristics	Y	42770203

[a] Y = Yes; N = No; N/A = Not Applicable. Data were submitted in support of an experimental use permit on cotton. Data requirements followed by MRID citations reflect conclusions determined in this document.

[b] The registrant must clarify which method is the current process proposed for production. If both methods are to be used, the registrant must determine if the two different processes produce basic and alternate formulations of the 94.5% T as defined under 40 CFR §152.43, and submit revised CSFs for the two formulations. In addition, the registrant must propose more appropriate certified limits for the ai (±3%; 40 CFR §158.175(b)(2)) or submit a justification for the proposed limits. The impurities must be listed on EPA Form 8570-4 (Rev. 12/90) by the commonly accepted chemical name.

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[c] The registrant must submit the duration of each step and of the entire manufacturing process, and clarify which method is proposed for production. If both processes are to be used, the registrant must determine if Method A and Method B produce basic and alternate formulations as defined under 40 CFR §152.43.

[d] A preliminary analysis study must be submitted on five representative batches of the final product manufactured by the process to be used for production. All components present at greater than or equal to 0.1% must be identified and quantitated, demonstrating good total closure.

[e] The stability of the TGAI on exposure to sunlight and to metals and metal ions must be submitted.

PIRATE (AMERICAN CYANAMID)

PRODUCT CHEMISTRY

TASK 4

(Final Report)

CONFIDENTIAL APPENDIX

24 Page(s)

Confidential Appendix to the Scientific Review for the pesticide Pirate by the Chemistry Branch Tolerance Support [Confidential FIFRA Trade Secret/CBI].

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CHLORFENAPYR

Page _____ is not included in this copy.

Pages 57 through 80 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

X Description of the product manufacturing process.

X Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

_____ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.