UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

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OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

Date: 11/16/05

Subject: Cloquintocet-mexyl on Wheat and Barley. Data Submitted to Satisfy Conditional Registration Requirements for Use on Wheat, Revise Wheat Tolerances, and to Add Use on Barley. Summary of Analytical Chemistry and Residue Data. PP#'s 7E04920 and 4E06831.

DP Number:	308470	Decision Number:	301711
PC Code:	700099	MRIDs:	46012904, 46012905, 46012909, 46012910, 46012911, 46012912, 46012913, 46012914, 46012915, 46012916, 46012917, 46012918, 46203138, 46203139,46203140, 46203142, 46203143, 46203203, 46203204, 46203205, 46203206, 46373301, 46373302
40 CFR:	180.560	Chemical Class:	Herbicide safener

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This document was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 12/15/2004). The document has been reviewed by HED and revised to reflect current OPP policies.

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Executive Summary

In PP#7E04920, Syngenta Crop Protection, Inc. proposed the establishment of tolerances for residues of the safener cloquintocet-mexyl (CGA-185072) in or on wheat grain at 0.02 ppm and wheat straw at 0.05 ppm. The safener is contained in the formulation Discover™ Herbicide, which contains the herbicide clodinafop-propargyl (CGA-184927). Cloquintocet-mexyl is needed in the formulation to prevent damage to the wheat due to phytotoxic effects of clodinafop-propargyl.

HED reviewed the data submitted in conjunction with the petition and concluded that, provided revised Sections B and F were submitted and the method validation was satisfactory, the submitted data were adequate to support a conditional registration for use of cloquintocet-mexyl on wheat (DP Barcode D257181, N. Dodd, 4/7/00). Tolerances were subsequently established for the combined residues of cloquintocet-mexyl (acetic acid, [(5-chloro-8-quinolinyl)oxy]-, 1-methylhexyl ester) and its acid metabolite (5-chloro-8-quinolinoxyacetic acid), when used as an inert ingredient (safener) in pesticide formulations containing the herbicide clodinafop-propargyl in a 1:4 ratio of safener to active ingredient, in/on the following raw agricultural commodities [40 CFR §180.560(a)]:

Wheat, forage	0.1 ppm
Wheat, grain	0.1 ppm
Wheat, hay	0.1 ppm
Wheat, straw	0.1 ppm

In the previous (4/7/00) review, HED concluded that prior to granting full registration, additional data and/or information were required regarding plant and livestock metabolism, plant analytical methods, multiresidue methods, storage stability, crop field trials, processing studies, and confined rotational crops.

Syngenta has now submitted data to address the requirements of conditional registration for cloquintocet-mexyl. (Data for the active ingredient clodinafop-propargyl are reviewed separately in D295198.)

Syngenta has also petitioned for amended tolerances to support additional use of cloquintocet-mexyl as a safener in pesticide formulations containing the new active ingredient pinoxaden for use on both wheat and barley. In connection with the pinoxaden petition, Syngenta proposed to revise the established tolerances for cloquintocet-mexyl residues in/on wheat commodities and proposed tolerances on barley commodities (PP#4E06831; 69 FR 31118, 6/2/04; and 69 FR 67731, 11/19/04).

Syngenta now proposes the following tolerances for the combined residues of cloquintocet-mexyl (acetic acid, [(5-chloro-8-quinolinyl)oxy]-, 1-methylhexyl ester) and its acid metabolite (5-chloro-8-quinolinoxyacetic acid) when used as an inert ingredient (safener) in pesticide

formulations containing either the herbicide clodinafop-propargyl or pinoxaden in a 1:4 ratio of safener to active ingredient in or on wheat and barley commodities as follows:

Wheat, forage	0.2
Wheat, straw	0.1
Wheat, hay	0.5
Wheat, grain	0.01
Barley, hay	0.1
Barley, straw	0.1
Barley, grain	0.01

Clodinafop-propargyl is a systemic herbicide that belongs to the oxyphenoxy acid ester chemical class. Two clodinafop-propargyl end-use products are conditionally registered for use on wheat: the 2 lb/gal emulsifiable concentrate (EC) formulation (Discover® Herbicide; EPA Reg. No. 100-907) and the 0.5 lb/gal EC formulation (Discover® NG Herbicide; EPA Reg. No. 100-1173). Currently, use of these products is restricted to Montana (MT), Minnesota (MN), North Dakota (ND), and South Dakota (SD). The petitioner wishes to expand use to wheat grown in all areas of the U.S.

Pinoxaden is a systemic herbicide. One pinoxaden end-use product is proposed for use on barley and wheat: Axial™ Herbicide (EPA Reg. No. 100-XXX), an EC formulation which contains 0.83 lb pinoxaden/gal.

The ratio of 1:4 safener to active ingredient applies to all three formulations (Discover® Herbicide, Discover® NG Herbicide, and Axial ™ Herbicide).

The nature of the residue in wheat, barley, livestock, and rotational crops is adequately understood. The HED Metabolism Assessment Review Committee (MARC) previously determined for the purpose of the conditional registration that the residues of concern for the tolerance expression and risk assessment for plants, livestock, and rotational crops are cloquintocet-mexyl and its metabolite CGA-153433 (D263289, N. Dodd, 2/25/00). HED concludes that the residues of concern for the tolerance expression and risk assessment in wheat, barley, livestock, and rotational crops are cloquintocet-mexyl and its metabolite CGA-153433, the residues that are currently regulated.

Adequate enforcement methods are available for enforcement of the proposed/existing tolerances on wheat and barley. The two enforcement methods are the high performance liquid chromatography/ultraviolet detection (HPLC/UV) method REM 138.01 for determination of cloquintocet-mexyl (parent) and the HPLC/UV Method REM 138.10 for determination of the metabolite CGA-153433. Adequate EPA petition method validations have been conducted on wheat grain, straw, and forage for the two enforcement methods (D262416, Elmer Hayes, Analytical Chemistry Branch/Biological and Economic Analysis Division (ACB/BEAD), 6/22/00; D267870, N. Dodd, 8/8/00). Both methods have been forwarded to FDA for publication in

Pesticide Analytical Methods (PAM), Vol. II. The validated limits of quantitation (LOQs) for Method REM 138.01 are 0.05 ppm for wheat forage, hay, and straw, and 0.02 ppm for wheat grain, processed commodities, and aspirated grain fractions. The validated LOQ for Method REM 138.10 is 0.05 ppm for all wheat commodities.

Because of the low levels of total radioactive residues found in livestock commodities in the ruminant and poultry metabolism studies and the corresponding low radioactive residues calculated for the 1X feeding levels, ruminant and poultry feeding studies are not needed and tolerances on livestock commodities are not needed. The uses on wheat and barley fall under 40 CFR §180.6(a)(3) since no secondary residues are expected to occur in livestock commodities.

The submitted wheat field trials are adequate for a conditional registration. Samples of wheat forage, hay, grain, and straw were analyzed. Most of the wheat samples were analyzed by the HPLC/UV enforcement methods REM 138.01 (for parent) and REM 183.10 (for the metabolite CGA-153433). The validated LOQs for Method REM 138.01 are 0.05 ppm for wheat forage, hay, and straw, and 0.02 ppm for wheat grain, processed commodities, and aspirated grain fractions. The validated LOQ for Method REM 138.10 is 0.05 ppm for all wheat commodities. Residues of parent and its metabolite CGA-153433 in spring wheat and winter wheat planted in the spring will not exceed 0.1 ppm in wheat grain, forage, hay, and straw.

The submitted barley field trials are adequate in number and geographic representation. The data collection methods for barley determined combined residues of cloquintocet-mexyl and its metabolite CGA-153433 as CGA-153433, using an oxidation step to convert cloquintocet-mexyl to CGA-153433. Based on these residue data, residues are not expected to exceed 0.010 ppm in barley grain (LOQ) and 0.050 ppm in barley hay and straw. Because the EPA-validated enforcement methods determine parent and metabolite separately at LOQs of 0.05 ppm for each analyte on each commodity except for an LOQ of 0.02 ppm for parent in grain, the tolerances for the combined residues of cloquintocet-mexyl and its metabolite CGA-1532433 should be 0.10 ppm for barley, hay; 0.10 ppm for barley, grain; and 0.10 ppm for barley, straw.

The submitted processing data for wheat are tentatively adequate to satisfy data requirements, pending submission of additional information requested for Study 300/91 (MRID 44399210) regarding the storage stability data for grain. The processing data indicate that residues of cloquintocet-mexyl and CGA-153433, determined as CGA-153433, do not concentrate in wheat processed commodities (bran, flour, middlings, shorts, and germ). Residues may concentrate in aspirated grain fractions (AGF) but residues in AGF are not likely to exceed the recommended tolerance of 0.10 ppm for grain. Therefore, tolerances are not needed for the wheat processed commodities (bran, flour, middlings, shorts, and germ) or for aspirated grain fractions.

Pending submission of additional information regarding storage stability of grain requested for Study 300/91 (MRID 44399210), HED tentatively concludes that the submitted barley processing study is adequate. Residues were <LOQ (<0.01 ppm) in barley grain treated at 5X and <LOQ (<0.01 ppm) in the processed fractions pearled barley, flour, and bran. Residues do not

concentrate on processing and tolerances on the processed commodities pearled barley, flour, and bran are not needed.

Based on the available confined rotational crop data, the proposed rotational crop restrictions are appropriate. No field rotational crop data have been submitted but none are needed to support the requested uses on wheat and barley.

There are currently no Codex, Canadian, or Mexican MRLs/tolerances established for cloquintocet-mexyl on wheat. Therefore, no compatibility questions exist.

Regulatory Recommendations

HED has examined the data submitted by the petitioner to satisfy the requirements for full registration and concludes that full registration for Discover® Herbicide and Discover® NG Herbicide on wheat may not granted until the deficiencies noted below have been resolved. The available data indicate that no revisions to the current tolerance level of 0.10 ppm on wheat, grain; wheat, forage; wheat, hay; and wheat, straw are needed.

Provided the petitioner submits a revised Section B for Axial™ Herbicide and a revised Section F, there will be no residue chemistry requirements that would preclude the establishment of a conditional registration for Axial™ Herbicide on wheat and barley and tolerances for the combined residues of cloquintocet-mexyl and its acid metabolite (5-chloro-8-quinolinoxyacetic acid) on barley, grain; barley, hay; and barley, straw at 0.10 ppm.

Residue Chemistry Deficiencies

860.1200 Directions for Use

1. The proposed Axial™ Herbicide label is adequate to allow evaluation of the residue data submitted in support of this petition provided that the following label restriction, which is already on the Discover®/Discover® NG labels, is added to the Axial label: "Do not apply to winter wheat in the fall."

2. Since residue data support one application per season, the Discover®/Discover®NG Herbicide labels and the Axial™ Herbicide labels should all contain the statement "Do not apply both Discover® and Axial™ products to the same crop in the same season."

860.1380 Storage Stability

3. The requirement from the 4/7/00 review for additional information to support Study 300/91 reported in MRID 44399210 (storage stability study for CGA-153433 in wheat grain) has not been addressed by the petitioner. The petitioner must submit the following additional data to support the storage stability study in MRID 44399210 for CGA-153433 in wheat grain: (1) raw data,

including residues (ppm) found and representative chromatograms (for standards, controls, freshly fortified samples, and stored samples); (2) description of storage containers; and (3) submission or identification by number of the method used to analyze the storage stability samples.

860.1500 Crop Field Trials

4. The wheat field trials are not adequate in number or geographic representation. Because the data for the winter wheat treated in the fall will not be used, the number and geographic representation for the wheat field trials are not adequate. An additional 8 field trials are needed for adequate geographic representation as follows: 1 trial in Region 2, 1 trial in Region 4, 1 trial in Region 5, 1 trial in Region 6, and 4 trials in Region 8. For Axial™ Herbicide, the petitioner should add the restriction "Do not apply to winter wheat in the fall" to the label; this restriction is already on the Discover®/Discover® NG Herbicide labels.

Alternatively, the winter wheat data could be used but the higher tolerances then needed on wheat commodities would mean that a ruminant feeding study would be needed or a livestock method for meat, fat, and milk including radiovalidation data and an independent laboratory validation would be needed. A validation of the livestock method by EPA's ACB/BEAD would also be needed. If a validated livestock enforcement method could be made available, tolerances for ruminant commodities, pending ChemSAC approval, could be set at the LOQ of the method.

860.1520 Processed Food/Feed

5. The submitted processing data for barley and wheat are tentatively adequate to satisfy data requirements, pending submission of additional information requested for Study 300/91 (MRID 44399210) regarding the storage stability data for grain (requested under 860.1380 Storage Stability).

860.1550 Proposed Tolerances

6. A revised Section F must be submitted to propose retention of the tolerance of 0.10 ppm for wheat, forage; wheat, grain; wheat, hay; and wheat, straw and to propose a tolerance of 0.10 ppm for barley, grain; barley, hay; and barley, straw for cloquintocet-mexyl and its acid metabolite (5-chloro-8-quinolinoxyacetic acid).

Background

As a result of a Syngenta proposal in PP#7E4920, tolerances were established for residues of the safener cloquintocet-mexyl in pesticide formulations (Discover® Herbicide/Discover NG® Herbicide) in wheat commodities. Additional data were required before a permanent registration for cloquintocet-mexyl in/on wheat commodities could be established (D257181, N. Dodd, 4/7/00). Additional data and/or information were required for plant and livestock metabolism, plant analytical methods, multiresidue methods, storage stability, crop field trials, processing studies,

and rotational crops. The present submission is a response to the review of D257181 (N. Dodd, 4/7/00).

Syngenta has also petitioned for amended tolerances to support additional use of cloquintocet-mexyl as a safener in a pesticide formulation (Axial™ Herbicide) containing the new active ingredient pinoxaden for use on both wheat and barley. In connection with the pinoxaden petition, Syngenta proposed to revise the established tolerances for cloquintocet-mexyl residues in/on wheat commodities and proposed tolerances on barley commodities (PP#4E06831; 69 FR 31118, 6/2/04; and 69 FR 67731, 11/19/04).

The chemical structure and nomenclature of cloquintocet-mexyl and its metabolite CGA-153433 and the physicochemical properties of the technical grade of cloquintocet-mexyl are presented in the tables below. A table of the chemical structures and nomenclature of metabolites identified in the submitted metabolism study is presented in Appendix I.

Table 1. Cloquintocet-mexyl Nomenclature.

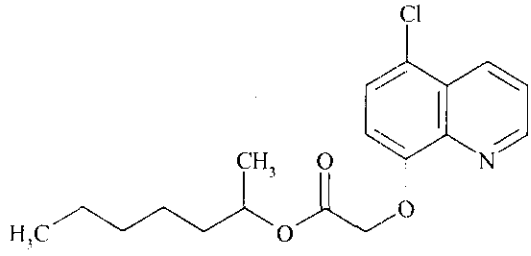
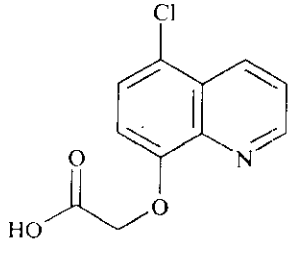
Chemical structure	
Common name	Cloquintocet-mexyl
Company experimental name	CGA-185072
IUPAC name	(5-chloroquinolin-8-yloxy)acetic acid 1-methylhexyl ester
CAS name	acetic acid, [(5-chloro-8-quinolinyl)oxy]-, 1-methylhexyl ester
CAS registry number	99607-70-2
End-use products (EPs)	Discover® Herbicide (EPA Reg. No. 100-907) Discover® NG Herbicide (EPA Reg. No. 100-1173) Axial™ Herbicide (EPA Reg. No. 100-XXX)
Chemical structure of cloquintocet-mexyl acid metabolite (CGA-153433)	 [(5-chloro-8-quinolinyl)oxy]acetic acid

Table 2. Physicochemical Properties of the Technical Grade Test Compound		
Parameter	Value	Reference
Melting range	61.4 to 69°C	MRID 44387401
pH	5.4 at 25°C (1% w/v aqueous disp.)	MRID 44387401
Density	1.05 g/cm^3 at 22°C	MRID 44387401
Water solubility (25°C)	0.59 mg/L at pH 7.0 (PAI)*	MRID 44387401
Solvent solubility (g/L) (25°C)	ethanol - 190 acetone - 340 toluene - 360 n -hexane - 0.140 n-octanol - 11	MRID 44387401
Vapor pressure at 25°C	3.98 x10^{-8} mm Hg (PAI)*	MRID 44387401
Dissociation constant (pK_a)	3.55 (PAI)*	MRID 44387401
Octanol/water partition coefficient log P_{ow}	5.03 (at 25°C) (PAI)*	MRID 44387401
UV/visible absorption spectrum	Two absorbance maxima occur at 243.8-255.8 nm and 317.6-364.0 nm. No absorbance maxima occur between 370 nm and 750 nm.	MRID 44387401

*PAI = pure active ingredient

860.1200 Directions for Use

The clodinafop-propargyl products Discover® Herbicide (EPA Reg. No. 100-907) and Discover® NG Herbicide (EPA Reg. No. 100-1173) are presently conditionally registered for foliar application to all varieties of wheat (including durum) grown in Montana, Minnesota, North Dakota, and South Dakota. The safener, cloquintocet-mexyl, is included for use as a component of Discover® Herbicide. The petitioner is now proposing to expand use of Discover® Herbicide and Discover® NG Herbicide to wheat grown in all areas of the U.S. and is addressing the conditions of registration. The petitioner is also requesting registration of the pinoxaden product Axial™ Herbicide for foliar application to wheat and barley.

Table 3. Summary of Directions for Use of Clodinafop-propargyl and Cloquintocet-mexyl.						
Trade Name	Applic. Timing; Type; and Equip.	Applic. Rate (lb ai A)	Max. No. Applic. per Season	Max. Seasonal Applic. Rate (lb ai/A)	PHI (days)	Use Directions and Limitations
Wheat (including Durum)						
Discover® Herbicide (EPA Reg. No. 100-907)[1] Discover® NG Herbicide (EPA Reg. No. 100-1173)	Postemergence, 2-leaf stage to pre-boot stage. Broadcast foliar Ground or aerial	0.050-0.062 clodinafop-propargyl 0.012-0.016 cloquintocet-mexyl	1	0.062 clodinafop-propargyl 0.016 cloquintocet-mexyl	60	Do not treat wheat underseeded to forages. Do not apply to winter wheat in the fall. Do not graze or feed forage or hay for 30 days after application. For rotational crops, the plantback intervals are 0 days for wheat (includling Duram) and 30 days for all other crops. Applications are to be made in a minimum spray volume of 3 gal/A for aerial equipment or 5 gal/A for ground equipment. Applications must always be made with DSV Adjuvant. Application through any type of irrigation system is prohibited. The restricted-entry interval (REI) is 12 hours.

[1] The Discover® Herbicide label is dated 5/29/03.

Table 4. Summary of Directions for Use of Pinoxaden and Cloquintocet-mexyl.						
Trade Name	Applic. Timing; Type; and Equip.	Applic. Rate (lb ai/A)	Max. No. Applic. per Season	Max. Seasonal Applic. Rate (lb ai/A)	PHI (days)	Use Directions and Limitations
Barley and Wheat (including Durum)						
Axial ™ Herbicide (EPA Reg. No. 100-XXX)	Postemergence, 2-leaf stage to pre-boot stage. Broadcast foliar Ground or aerial	0.036 - 0.062 pinoxaden 0.009-0.016 cloquintocet-mexyl	1	0.062 pinoxaden 0.016 cloquintocet-mexyl	60	Do not graze livestock or feed forage or hay for 30 days after application. Do not treat wheat or barley underseeded to forages. For rotational crops, the plantback intervals are 0 days for barley and wheat (including Duram), 30 days for leafy crops and root crops, and 120 days for other cereal grains and all other crops. Applications are to be made in a minimum spray volume of 3 gal/A for aerial equipment or 5 gal/A for ground equipment. Add the correct amount of Adigor Adjuvant to the spray tank. Application through any type of irrigation system is prohibited. The restricted-entry interval (REI) is 48 hours.

Deficiency #2 from D257181:

In the previous review for cloquintocet-mexyl (D257181, N. Dodd, 4/7/00; Deficiency 2), the following deficiency was noted:

> The Section B/label should be revised to change the feeding/grazing restriction on forage to 30 days since limited residue data are available at a 7-day PHI. Provided the above revision to the Section B/label is made, the proposed use of cloquintocet-mexyl on wheat will be adequately described. The proposed use directions will be adequate to allow an assessment of whether the residue data reflect the maximum residues likely to occur in food/feed.

Petitioner's Response to Deficiency #2:

In the Discover Herbicide label dated 5/29/03, the petitioner has modified the label accordingly.

Conclusions: Deficiency #2 from D257181 is resolved by submission of the revised label dated 5/29/03 to change the feeding/grazing restriction on forage to 30 days.

The Discover®/Discover® NG Herbicide labels are adequate to allow evaluation of the residue data submitted in support of this petition.

The proposed Axial™ Herbicide label is adequate to allow evaluation of the residue data submitted in support of this petition provided that the following label restriction, which is already on the Discover®/Discover® NG labels, is added to the Axial label: "Do not apply to winter wheat in the fall."

860.1300 Nature of the Residue - Plants

46012909.der.wpd

Deficiencies #'s 3a, 3b, and 3c from D257181:

In the previous review [D257181, N. Dodd, 4/7/00; Deficiency 3 (a, b, c)], HED requested the following additional information pertaining to the [3-^{14}C-quinoline]CGA-185072 wheat metabolism study:

> a) Due to large amounts of the radioactivity being nonextractable with 80% aqueous acetonitrile and by Soxhlet extraction with 100% acetonitrile, only 16.0%, 0.9%, and 4.4% total radioactive residues (TRR) were identified in leaves (ear emergence), leaves (milky stage), and straw (maturity), respectively. The petitioner should have attempted to extract more of the radioactivity using acid, base, and enzymes and then characterized/identified those residues.

b) Residues in grain were not identified in the field study. The identity of residues in grain resulting from application to the plant in a manner simulating expected field use are needed. The study should be conducted at a higher rate than the 2X study which was submitted.

c) The time from sampling to final analysis should be clarified for the wheat samples. If the time between sampling and final analysis of the field samples exceeded 6 months, evidence should be provided that the identity of residues did not change during the period between collection and final analysis. Such evidence would be analyses of representative substrates early in the study and at its completion. To be acceptable, such analyses should show that the basic profile of radiolabeled residues has not changed during that time.

Petitioner's Response to Deficiencies #3a, 3b, and 3c from D257181:

In response, Syngenta has submitted a study investigating the metabolism of [quinoline-3-^{14}C]cloquintocet-mexyl in spring wheat:

Wheat Metabolism Study (MRID 46012909)

Syngenta has submitted a study (MRID 46012909) investigating the metabolism of [quinoline-3-^{14}C]cloquintocet-mexyl (specific activity 55.2 µCi/mg) in spring wheat. The radiolabeled test substance was formulated as an EC formulation, mixed with Score® adjuvant and non-labeled clodinafop-propargyl (herbicide) and then diluted with water and applied to wheat plants, as a single foliar treatment, at 0.016 or 0.16 lb safener/A (1x or 10x the proposed maximum seasonal rate). Applications were made to wheat plants at BBCH growth stages 22-30. Wheat forage was harvested 7 and 30 days after treatment, and wheat grain and straw were harvested at maturity, 61 days following treatment.

In wheat plants treated with [^{14}C]cloquinotcet-mexyl at 1x, total radioactive residues (TRR) were 0.438 ppm in forage harvested 7 days posttreatment, 0.019 ppm in forage harvested 30 days posttreatment, 0.004 ppm in mature straw, and 0.003 ppm in mature grain. In wheat plants treated with [^{14}C]cloquinotcet-mexyl at 10x, TRR were 3.004 ppm in forage harvested 7 days posttreatment, 0.204 ppm in forage harvested 30 days posttreatment, 0.029 ppm in mature straw, and 0.012 ppm in mature grain. Because of low radioactivity levels, samples of mature straw and grain from the 1x treatment were not subjected to extraction procedures. The majority of the TRR (~50-73%) was extracted from wheat forage using acetonitrile (ACN)/water; ACN/water released smaller amounts (29-35% TRR) from straw and grain. The petitioner subjected the ACN/water extract to cellulase hydrolysis and compared the chromatographic profile with that of the extract prior to hydrolysis. The nonextractable residues of 7-day PHI forage (both treatments) and 30-day PHI forage (10x treatment), which accounted for 29-36% TRR, were subjected to sequential microwave hydrolyses, using isopropanol/water, 0.5 N HCl, and 0.5 N NaOH to release additional residues, yielding 7-15% TRR in the isopropanol/water hydrolysate, 5-13% TRR in the 0.5 N HCl hydrolysate, and 6-14% TRR in the 0.5 N NaOH hydrolysate. The nonextractable residues of 30-day PHI forage (1x treatment) and mature straw and grain (10x treatment) accounted for 0.006-

0.017 ppm and were not subjected to additional characterization attempts. The accountabilities were 92-108%.

Residues were characterized/identified primarily by two-dimensional thin layer chromatography (TLC) analyses, with confirmatory analysis by HPLC. Liquid chromatography/mass spectrometry (LC/MS) and ^{1}H-nuclear magnetic resonance (NMR) analyses were used to identify the two major metabolites in 7-day PHI forage (10x treatment). These methods successfully identified the predominant residues in wheat matrices. Adequate storage stability data were submitted, demonstrating the stability of the metabolite profiles in wheat forage for up to 26 months. Wheat grain and straw samples were analyzed within 3 months of sample collection; therefore, supporting storage stability data are not needed for these matrices.

Approximately 31-39% TRR were identified in forage, and 7% TRR were identified in mature straw. No metabolites were identified in mature wheat grain. Cloquintocet-mexyl was found in minor amounts in 7-day PHI forage only, at 2.2% and 3.4% TRR (0.015 and 0.066 ppm). The metabolite OH-CGA-153433 was found to be the major metabolite, accounting for a total of 20.5-22.5% TRR (0.042-0.676 ppm) in forage (7-day PHI, both treatment rates, and 30-day PHI, 10x treatment) and 7.3% TRR (0.002 ppm) in mature straw (10x treatment). Of these amounts, 11.9-12.9% TRR (0.026-0.388 ppm) was conjugated OH-CGA-153433 in forage (released after hydrolysis with cellulase). Metabolite CGA-153433 accounted for a total of 2.7-10.5% TRR (0.006-0.276 ppm) in forage; of these amounts, ~7% TRR was conjugated in 7-day PHI forage. Hydrolysis of bound residues yielded small amounts of CGA-153433 (1.8-3.7% TRR) and OH-CGA-153433 (1.6-4.4% TRR) in forage. A large portion of the remaining radioactivity consisted of unknowns; HPLC analyses of extracts and hydrolysates indicated that unknown peaks were each ≤4.6% TRR (≤0.020 ppm) in 1x treatment 7-day PHI forage, ≤5.0% TRR (≤0.150 ppm) in 10x treatment 7-day PHI forage, ≤3.7% TRR (≤0.008 ppm) in 10x treatment 30-day PHI forage, and ≤0.003 ppm in 10x treatment mature straw.

Based on the results of this study, the petitioner concluded that metabolism of cloquintocet-mexyl is rapid, since low levels of parent were observed in 7-day PHI forage and parent was not detected in 30-day PHI forage or mature grain and straw. Cloquintocet-mexyl undergoes de-esterification to form CGA-153433 which is hydroxylated to form OH-CGA-153433. Further metabolism results in the binding of these metabolites to crop matrix and the generation of multiple polar components.

Conclusions. Deficiencies #'s 3a, 3b, and 3c from D257181 are resolved by submission of the wheat metabolism study (MRID 46012909), which is adequate to satisfy data requirements.

Deficiency #4 from D257181:

> The residues of concern in wheat were determined by HED's Metabolism Assessment Review Committee (MARC) on 2/15/00 (D263289, N. Dodd, 2/25/00) to be CGA-185072 and its acid metabolite CGA-153433. HED may revisit the MARC decision after additional wheat metabolism data have been submitted.

Discussion: In the submitted wheat metabolism study (MRID 46012909), OH-CGA-153433 was found as a major metabolite in forage. Although OH-CGA-153433 may be found in forage, the amount in the metabolism study extrapolated to 1X at the 30-day PHI is less than 0.005 ppm. Considering the polarity of this metabolite (COOH and OH groups) and that a substantial portion is conjugated, significant residues would not be expected to occur in livestock commodities. Therefore. HED does not consider OH-CGA-153433 to be a residue of concern in wheat forage.

Conclusion: Deficiency #4 is resolved.. HED concludes that the residues of concern for the tolerance expression and risk assessment are cloquintocet-mexyl and its metabolite CGA-153433, the residues that are currently regulated (40 CFR §180.560).

860.1300 Nature of the Residue - Livestock

46373301.der.wpd
46373302.der.wpd

Deficiencies #'s 5 and 7 from D257181:

In the previous review (D257181, N. Dodd, 4/7/00; Deficiencies 5 and 7), HED requested the following additional information regarding the goat and hen metabolism studies:

> 5. Ruminants: The nature of the residue in ruminants is not adequately understood for the purpose of a permanent tolerance for the following reason: The time between sampling and final analysis should be clarified for milk and tissues. If the time between sampling and final analysis of the samples exceeded 6 months, evidence should be provided that the identity of residues did not change during the period between collection and final analysis. Such evidence would be analyses of representative substrates early in the study and at its completion. To be acceptable, such analyses would show that the basic profile of radiolabeled residues has not changed during that time.
>
> 7. Poultry: The nature of the residue in poultry is not adequately understood for the purpose of a permanent tolerance for the following reason: The time between sampling and final analysis should be clarified for eggs and tissues. If the time between sampling and final analysis of the samples exceeded 6 months, evidence should be provided that the identity of residues did not change during the period between collection and final analysis. Such evidence would be analyses of representative substrates early in the study and at its completion. To be acceptable, such analyses would show that the basic profile of radiolabeled residues has not changed during that time.

Petitoner's Response to Deficiencies #'s 5 and 7:

In response, the petitioner submitted new goat and hen metabolism studies:

Goat Metabolism Study (MRID 46373301)

[Quinolinyl-3-^{14}C]CGA-185072 (cloquintocet-mexyl, 99.6% radiochemical purity, 1.462 MBq/mg) was administered by gavage to two lactating Alpine goats once/day for 4 days at a concentration of ~4.3 mg/kg bw/day (126 ppm in the diet). Milk was collected twice daily, and urine, feces, and cage wash were collected daily at 24-hour intervals until sacrifice 6 hours after the last dose. Muscle, fat, liver, kidney and pooled bile were collected at sacrifice. The excreta, bile, milk, and tissue samples were extracted with acetonitrile and/or acetonitrile:water and analyzed chromatographically for radioactive components. Based on HPLC profiling of the extracts near the beginning and end of the study, the residues in the extracts were reasonably stable during the study.

Overall recovery of the radiolabel was adequate, with a mean recovery of 88% of the radioactivity administered to the two goats. Most of the radioactivity was excreted in the urine and feces. Mean recoveries from the two goats of the total dose administered were 0.2% of the dose in milk, ≤0.1% of the dose in each tissue (muscle, fat, kidney, and liver), 23% in the gastrointestinal (GI) tract, 52% in urine, and 12% in feces.

Tissue samples collected from the two goats six hours after the final dose contained mean residues of 0.017 ppm CGA-185072 equivalents in the leg muscle, 0.037 ppm in the fat, 0.214 ppm in the liver, and 2.907 ppm in the kidney. Mean residues in milk were 0.158-0.485 ppm (study avg 0.30 ppm) CGA-185072 equivalents.

The major metabolite recovered in the milk, tissues, bile, and excreta was CGA-153433, the ester hydrolysis product of CGA-185072. This metabolite accounted for 51.9% TRR in the liver, 53.4% TRR in the muscle, 67.1% TRR in the fat, 74.7% TRR in the kidney, and 80.2% TRR in the milk. Two other minor metabolites were identified. Small amounts of the metabolite M-2 were found in kidney, liver, muscle, and fat (≤5% TRR per matrix). Metabolite M-2 was formed through intramolecular cyclization, hydroxylation para to the nitrogen and reduction of the pyridine ring of CGA-185072. This metabolite was then conjugated to form the glucuronide metabolite known as M-1. Metabolite M-1 was found in kidney, liver, muscle, fat, and milk (≤11% TRR per matrix). Small amounts of parent were found in the kidney, liver, muscle, and fat (≤9.5%).

Poultry Metabolism Study (MRID 46373302)

[Quinolinyl-3-^{14}C]CGA-185072 (cloquintocet-mexyl, 99.6% radiochemical purity) was administered once per day orally to five white leghorn laying hens for 8 consecutive days at a feeding level of 7.4 mg/kg bw/day (95 ppm in the diet). Excreta and eggs were collected daily. Six hours after the final dose, the hens were sacrificed and the blood, skin with attached fat, thigh and breast muscle, peritoneal fat, and liver were collected. The residual carcasses were not examined. Muscle, liver, and egg yolks were extracted in acetonitrile/water (8:2). Egg white was extracted with acetonitrile. Fat was extracted with acetonitrile:hexane (2:1), by first stirring the fat in warm hexane and then adding the acetonitrile. Neutral solvent extractability was variable and ranged from 36% in the muscle to 87% in the fat. The excreta were not extracted. Enzyme hydrolysis of the muscle, liver, and egg yolk released most of the remaining radiolabeled residues. The neutral

extracts were shown to be reasonably stable, based on high performance liquid chromatography (HPLC) profiling conducted 0-44 days after extraction and again 553-678 days after the initial profiling.

The majority of the dose (66%) was recovered in the excreta. Small amounts of the dose were recovered in tissues and eggs: 0.02% of the dose was recovered in the muscle and peritoneal fat, 0.04% was recovered in the liver, 0.01% was recovered in the egg white, and <0.01% was recovered in the egg yolk.

Total radioactive residues were 0.010 ppm in muscle, 0.146 ppm in liver, 0.070 ppm in fat, 0.024-0.042 ppm in egg whites, and 0.002-0.018 ppm in egg yolks.

A large majority of the radioactivity was associated with the ester hydrolysis product CGA-153433 (5-chloro-8-quinolinoxyacetic acid). This metabolite comprised 73.3% TRR in the fat, 64.9% TRR in the liver, 78.0% TRR in the egg white, 50.5% TRR in the egg yolk, and 50.5% TRR in the muscle. One other metabolic product was identified in the liver, that being the hydroxylated product of CGA-153433 (10.2% TRR).

Conclusions. Deficiency #5 regarding the nature of the residue in ruminants is adequately resolved by submission of the new goat metabolism study (MRID 46373301). Deficiency #7 regarding the nature of the residue in poultry is adequately resolved by submission of the new poultry metabolism study (MRID 46373302).

The nature of the residue in ruminants and poultry is adequately understood. The HED Metabolism Assessment Review Committee (MARC) previously determined for the purpose of the conditional registration that the residues of concern for the tolerance expression and risk assessment for livestock are cloquintocet-mexyl and its metabolite CGA-153433 (D263289, N. Dodd, 2/25/00). HED concludes that the residues of concern in ruminants and poultry are the parent CGA-185072 and its acid metabolite CGA-153433.

860.1340 Residue Analytical Methods

46012910.der.wpd (also includes review of MRID 46012915)
46012912.der.wpd (also includes review of MRID 46012913 and 46012914)
46203138.der.wpd (also includes 46203139, 46203140, 46203142, and 46203143)
MRID 44399211

Wheat

Deficiencies #'s 9 (a and b), 10 (a, b, c, and d), and 11 from D257181:

In the previous review (D257181, N. Dodd, 4/7/00; Deficiencies 9, 10, and 11), HED requested additional information regarding the residue analytical methods for plants:

9. The following additional information is needed regarding the plant analytical methods used to obtain the storage stability and residue data: a) Radiovalidation data for Methods REM 138.01, 138.06, 138.10, and 138.12 are needed to demonstrate the efficiency of the methods in extracting and quantifying aged or bound residues in samples; b) Method REM 138.12 should be submitted.

10. The following additional information is needed for the proposed enforcement methods: a) For REM 138.01 and REM 138.06, either an interference study must be submitted which determines whether other pesticides registered on wheat will interfere with the analysis of cloquintocet-mexyl residues by the enforcement method or a specific confirmatory method such as mass spectroscopy is needed as discussed in OPPTS GLN 860.1340. Provided that a specific confirmatory method is available, the Agency will not require that an interference study be conducted; b) Confirmatory methods are needed for REM 138.01 and 138.06; c) The GC/MS confirmatory method in Method REM 138.10 includes derivatization with diazomethane. The petitioner should investigate whether another methylating agent could be substituted for diazomethane. If an alternative methylating agent is not available, EPA requires that justification for the use of diazomethane be provided. An alternative confirmatory method for REM 138.10 would be LC/MS. REM 138.10 could be rewritten to include LC/MS as the confirmatory method instead of GC/MS; d) Adequate EPA petition method validations are needed for the proposed enforcement methods. RAB3 has requested EPA petition method validations for REM 138.01, 138.06, and 138.10. These EPA petition method validations are underway. Adequate independent laboratory validations have been provided for methods REM 138.01 and 138.06.

11. Provided that the petition method validations which are being conducted by EPA are successful, adequate enforcement methods are available to enforce the tolerances on wheat.

Petitioners's Response to Deficiency # 9a from D257181:

In response to the 4/7/00 review, Syngenta has submitted radiovalidation data for Methods REM 138.01, 138.06, 138.10 REM, and Method REM 138.12 (MRIDs 46012912-46012915). The samples used for the study had been treated with [quinoline-3-^{14}C]cloquintocet-mexyl at 0.157 lb safener/A. The petitioner noted that, in the metabolism studies, no residues of cloquintocet-mexyl (parent) were found in mature grain and straw following treatment with [^{14}C]cloquintocet-mexyl. In the samples used for radiovalidation, residues of CGA-153433 were identified at 0.010 ppm in 30-day PHI wheat forage. This level is below the LOQ of Methods 138.06 and 138.10 for wheat forage (0.05 ppm for forage).

Although the low residue levels in metabolism samples prevented radiovalidation of the full method, the petitioner generated extraction efficiency data by subjecting wheat samples from the metabolism studies to the extraction procedures of Method REM 138.01 (grain and straw), Method REM 138.06 (grain and straw), Method REM 138.10 (forage, grain, and straw), and Method REM 138.12 (forage, grain, and straw). Based on the amount of radioactivity extracted, the extraction

efficiency data indicate that Methods REM 138.06 and REM 138.10 would adequately extract incurred residues of CGA-153433 from wheat forage, straw, and grain samples. The extraction efficiency data submitted for Method REM 138.01 and Method REM 138.12 indicate that the method extraction procedures do not release as much radioactivity as was released by the extraction procedures used in the metabolism studies. However, the metabolism study data indicate that no significant residues of cloquintocet-mexyl would be expected in wheat commodities. Method REM 138.12 is not proposed for enforcement.

Conclusion 9a:

Deficiency #9a has been resolved by submission of radiovalidation data for the extraction methods of Methods 138.01, 138.06, 38.10, and 138.12. Based on the amount of radioactivity extracted, the extraction efficiency data indicate that Methods REM 138.06 and REM 138.10 would adequately extract incurred residues of CGA-153433 from wheat forage, straw, and grain samples. The extraction efficiency data submitted for Method REM 138.01 indicate that the method extraction procedures do not release as much radioactivity as was released by the extraction procedures used in the metabolism studies. However, the metabolism study data indicate that no significant residues of cloquintocet-mexyl would be expected in wheat commodities. The petitioner should note that if additional uses of cloquintocet-mexyl are proposed in the future which result in measurable residues of concern in food/feed commodities, then additional radiovalidation data would be required for Method REM 138.01, the enforcement method for parent. Method REM 138.12, a minor modification of Method REM 138.01, is not proposed for enforcement; therefore, additional data are not required for Method REM 138.12.

Petitioner's Response to Deficiency # 9b from D257181:

In the 4/7/00 review, analytical method REM 138.01 was used to determine cloquintocet-mexyl residues in wheat in all of the crop field trial and processing study samples from the U.S. trials and in some of the storage stability study samples; method REM 138.06 was used to determine CGA-153433 in wheat in some storage stability study samples; and method REM 138.10 was used to determine CGA-153433 residues in wheat in all of the crop field trial and processing study samples from the U.S. trials and in some of the storage stability study samples. Analytical method REM 138.12, a minor improvement of REM 183.01, was used to determine cloquintocet-mexyl in some wheat storage stability study samples. HED required that method REM 138.12 be submitted to EPA.

Syngenta has now submitted a description of Method REM 138.12 for the determination of residues of cloquintocet-mexyl (parent) in/on wheat forage, grain, and fodder (straw; MRID 46012910). We note that the method also includes instructions for determination of residues in soil; information pertaining to soil will not be discussed in this review.

Briefly, samples are extracted with acetonitrile (ACN), and fatty co-extractables are removed by partitioning with hexane. The extract is cleaned up by C-18 solid-phase extraction (SPE), followed by re-extraction into hexane/diethyl ether and further cleanup by silica solid phase extraction

(SPE). Cloquintocet-mexyl (parent) is then determined by HPLC/UV with column switching. The reported LOQs were 0.02 ppm for wheat grain and 0.05 ppm for wheat straw and forage, based on the lower limit of method validation.

Method validation data for Method REM 138.12 demonstrated adequate method recoveries of cloquintocet-mexyl (parent) at the LOQ and 5x the LOQ for wheat grain, and at 2x the LOQ and 20x the LOQ for wheat forage and straw. No validation data at the LOQ for wheat straw and forage were included. For cloquintocet-mexyl, recoveries were 72-91% (7.9%) in wheat grain and were 68-106% (11%) in wheat forage and straw.

Conclusion 9b:

Deficiency #9b is resolved since the petitioner submitted a description of Method REM 138.12 for the determination of residues of cloquintocet-mexyl in/on wheat forage, grain, and fodder (straw) in MRID 46012910.

Petitioners's Response to Deficiency # 10 (a, b, and c) from D257181:

To satisfy the requirements of the 4/7/00 review, the petitioner has also submitted confirmatory methods for cloquintocet-mexyl and its metabolite CGA-153433 (MRID 46012911). The petitioner stated that residue identification of cloquintocet-mexyl may be confirmed by HPLC/MS/MS analysis of the extract resulting from sample analysis using Method REM 138.01, and that identification of CGA-153433 may be confirmed by HPLC/MS analysis of the extract resulting from Method REM 138.10. For cloquintocet-mexyl, HPLC/MS/MS analysis is conducted using an RPAQUEOUS-3 column, a gradient mobile phase of water and ACN, each containing 0.1% acetic acid, and MS/MS detection with positive ion Z-spray ionization, monitoring the product ion at m/z 237.9. For CGA-153433, HPLC/MS analysis is conducted using a C-18 column, a gradient mobile phase of water and ACN, each containing 0.1% acetic acid, and MS detection with positive ion electrospray ionization, monitoring the ion at m/z 238; if matrix interference is observed, MS detection may be conducted using negative ion electrospray ionization, monitoring the ion at m/z 236. These confirmatory methods were used in conjunction with the analysis of samples reported in MRID 46012904.

Data collection methods: The existing enforcement methods were used for data collection in the studies reported in this document. Samples of wheat forage and hay from the storage stability study, samples of wheat forage, hay, grain, and straw from the crop field trials, and samples of wheat grain, processed commodities, and aspirated grain fractions from the processing study were analyzed for residues of cloquintocet-mexyl using HPLC/UV method REM 138.01. The validated LOQs were 0.05 ppm for wheat forage, hay, and straw, and 0.02 ppm for wheat grain, processed commodities, and aspirated grain fractions. Samples of wheat forage, hay, and germ from the storage stability study, samples of wheat forage, hay, grain, and straw from the crop field trials, and samples of wheat grain, processed commodities, and aspirated grain fractions from the processing study were analyzed for residues of the metabolite CGA-153433 using HPLC/UV Method REM

138.10, or a modified version of the method in which CGA-153433 was detected and quantified using MS instead of UV. The validated LOQ was 0.05 ppm for all wheat commodities.

Conclusions 10a, 10b, and 10c:

Deficiency #10a has been resolved by submission of specific confirmatory methods in MRID 46012911: HPLC/MS/MS for confirmation of cloquintocet-mexyl and HPLC/MS for confirmation of CGA-153433.

Deficiency #10b has been resolved by submission of a confirmatory method in MRID 46012911 for analysis of cloquintocet-mexyl. The petitioner stated that the identification of cloquintocet-mexyl may be confirmed by HPLC/MS/MS analysis of the extract resulting from sample analysis using Method REM 138.01. A confirmatory method for Method REM138.06 was not submitted but will not be required because Method REM 138.06, which determines the metabolite CGA-153433, is not an existing enforcement method.

Deficiency #10c has been resolved by submission of a confirmatory method in MRID 46012911 for analysis of the metabolite CGA-153433 which does not use diazomethane. Identification of CGA-153433 may be confirmed by HPLC/MS analysis of the extract resulting from Method REM 138.10.

Discussion regarding Deficiencies #'s 10d and 11 from D257181:

Since the 4/7/00 review was written, adequate EPA petition method validations have been conducted on wheat grain, straw, and forage for two enforcement methods: Method REM 138.01 for determination of residues of the parent cloquintocet-mexyl and method REM 138.10 for determination of the metabolite CGA-153433 (D262416, E. Hayes, ACB/BEAD, 6/22/00; D267870, N. Dodd, 8/8/00). Both methods have been forwarded to FDA for publication in PAM, Vol. II (DP Barcode D271447, 4/2/02, N. Dodd; and memo dated 4/2/02 from N. Dodd to M. Wirtz). ACB/BEAD concluded that the LOQs reported by the registrant were adequate. The LOQs for REM 138.01 are 0.05 ppm for parent cloquintocet-mexyl in/on wheat forage, hay, and straw and 0.02 ppm for parent cloquintocet-mexyl in/on wheat grain and wheat processed commodities. The LOQs for REM 138.10 are 0.05 ppm for the metabolite CGA-153433 in/on wheat forage, hay, and straw and 0.02 ppm for CGA-153433 in/on wheat grain and wheat processed commodities.

Conclusions #10d and 11:

Deficiencies #'s 10d and 11 have been resolved since adequate EPA petition method validations have been conducted on wheat grain, straw, and forage for two enforcement methods: Method REM 138.01 for determination of residues of the parent cloquintocet-mexyl and method REM 138.10 for determination of the metabolite CGA-153433 (D262416). EPA validation of Method 138.06 as an enforcement method is not required; the method is adequate as a data collection

method. Adequate enforcement methods for the parent (REM 138.01, MRID # 44399211) and the metabolite CGA-153433 (REM 138.10, MRID 44755302) are available.

Additional Wheat and Barley Analytical Methods

Syngenta submitted analytical methods REM 199.02, REM 199.03, and 117-01 for analysis of residues of CGA-153433, the metabolite of cloquintocet-mexyl, in cereal grain matrices. Method REM 199.02 was used to determine residues of CGA-153433 in barley grain, hay, and straw in one barley field trial study (MRID 46203205) and in wheat field trials conducted in Canada (MRID 46302206). Method 117-01 was used to determine residues of CGA-153433 in barley grain, hay, and straw in one barley field trial study (MRID 46203204) and in the barley grain and processed commodities in the processing study (MRID 46203204). The methods also determine residues of pinoxaden in cereal matrices (DP Barcode 299651, Mohsen Sahafeyan, 7/7/05). All three methods possessed the same extraction procedure consisting of acid hydrolysis (1N HCl) by boiling under reflux for two hours. The acid hydrolysis is intended to convert the parent cloquintocet-mexyl (CGA-185072) to the acid metabolite, CGA-153433; however, validation/recovery data for CGA-185072 were not provided.

Method REM 199.02 (MRID 46203139) was proposed as a data-gathering method. The method involved extracting homogenized crop samples with 1N HCl under reflux for 2 hours. Following filtration (if necessary), the pH of the extract was raised by addition of 3% ammonia solution and the solution was allowed to settle for half an hour. The analysis of the resulting extract was performed by reversed-phase HPLC using a column-switching system connected via a pneumatically and thermally assisted electrospray ionization (ESI) to a tandem mass spectrometer (HPLC/HPLC-MS/MS). The LOQ of the method was reported as 0.02 ppm in cereal whole plants, ears, stalks and straw and as 0.01 ppm in cereal grains for CGA-153433. No limit of detection (LOD) was established in MRID 46203139. This method was found to give good recoveries within the acceptable range of 70-120% for the analysis of CGA-153433 in all the wheat matrices (whole plant, straw & grain) when spiked at the LOQ (0.01 ppm for grain and 0.02 ppm for all other matrices) and at 10X LOQ levels. The standard deviations (ranging from 1% to 12%) measured with respect to recoveries at each spiking level were indicative of the method having satisfactory repeatability. Good linearity was observed in the range of 0.35 to 20 ng/mL for CGA-153433 ($r^2 = 0.9996$). No independent laboratory validation (ILV) of this method was submitted. Based on the submitted recovery data for CGA-153433, analytical method REM 199.02 is acceptable as a data-gathering method for CGA-153433 in cereal matrices.

Method REM 199.03 (MRID 46203143) was proposed as a data-gathering method. The method involved extracting homogenized crop samples with 1N HCl under reflux for 2 hours. The pH of the extract was raised by addition of 3% ammonia solution (pH 3-4). The extract was centrifuged, filtered using a Vectaspin filtration tube and cleaned up on an Oasis HBL solid-phase extraction (SPE) cartridge eluted with dichloromethane:ethyl acetate:formic acid (80:20:0.5, v:v:v). The eluate was evaporated in the presence of 1N HCl solution and diluted with water prior to final analysis by HPLC-MS/MS. The LOQ of the method for CGA-153433 was reported as 0.02 ppm in cereal whole plants, ears, stalks and straw and as 0.01 ppm in cereal grains and cereal process

fractions. The LOD was estimated at 0.002 ppm for CGA-153433 in the matrices tested. Individual recoveries for CGA-153433 from Method REM 199.03 validation were all within the acceptable range of 70-120% for the analysis of barley whole plant samples at spiking levels of 0.01 ppm (n=5 at each spiking level) and 0.1 ppm. Individual recoveries for barley grain samples spiked with CGA-153433 at levels of 0.01 ppm (LOQ) and 0.5 ppm were within 70-120% except for one value (68%). Recoveries for barley straw spiked with CGA-153433 at 0.01 ppm and 0.1 ppm were generally within 70-120% except for two values (58% and 62%). The standard deviations (ranging from 3% to 8%) measured with respect to recoveries at each spiking level were indicative of the method having satisfactory repeatability. Good linearity was observed in the range of 0.00125 to 0.5 µg/mL for CGA-153433; r^2 = 0.9993 (grain), 0.9998 (straw), and 0.9991 (whole plant). No ILV of this method was submitted. Based on the submitted recovery data for CGA-153433, Method REM 199.03 is acceptable as a data-gathering method for CGA-153433 in cereal matrices.

Method REM 117-01 (MRID 46203138) is a proposed enforcement method. The method involved extracting homogenized crop samples with 1N HCl (or 1N HCl:acetonitrile (90:10, v:v)) under reflux for 2 hours. For determination of CGA-153433, an aliquot of the extract was filtered (if the solution was not clear) and after dilution with water, the final fraction was injected onto a reversed-phase C_{18} to ODS-3 two-column switching HPLC-MS/MS system for analysis. The C_{18} column was eluted with formic acid aqueous solution (0.1%):methanol (75:25, v:v) and the ODS-3 column with formic acid aqueous solution (0.05%):methanol (50:50, v:v). The LOQ of the method for CGA-153433 was reported as 0.02 ppm in cereal forage, hay and straw and as 0.01 ppm in cereal grains. The LOD of the method was reported as 0.00125 ng for CGA-153433. Syngenta Method 117-01 was found to give good recoveries within the acceptable range of 70-120% for the analysis of CGA-153433 in all the cereal matrices (grain, forage, hay & straw) when spiked at the LOQ (0.01 ppm for grain and 0.02 ppm for all other matrices) and up to ~1 ppm. The standard deviations (ranging from 2% to 10%) measured with respect to recoveries at each spiking level were indicative of the method having satisfactory repeatability. Good linearity was observed in the range of 0.00005 to 0.003 ng/µL for CGA-153433 (r^2 = 0.999889). The independent laboratory, EN-CAS Analytical Laboratories, successfully validated Method 117-01 in wheat (forage, straw, grain and aspirated grain fractions) and barley (hay and grain) matrices. The method was able to extract CGA-153433 and quantitate it at the expected range. Based on the submitted recovery data for CGA-153433 and the ILV results, Syngenta Method 117-01 is acceptable as a data-gathering method for CGA-153433 in cereal matrices. To be an enforcement method for cloquintocet-mexyl, EPA's analytical chemistry laboratory (ACB/BEAD) would have to validate the Method 117-01 for cloquintocet-mexyl (CGA-185072) and its metabolite CGA-153433 in cereal matrices and radiovalidation data for the method would have to be submitted.

Conclusions. HED concludes that residue analytical method data requirements are satisfied for wheat and barley commodities. All data requirements pertaining to residue analytical methods specified in the 4/7/00 review D257181 have now been satisfied.

Adequate enforcement methods are available for enforcement of the proposed/existing tolerances on wheat and barley. The two enforcement methods are the HPLC/UV method REM 138.01 for

determination of cloquintocet-mexyl (parent) and the HPLC/UV Method REM 138.10 for determination of the metabolite CGA-153433. Adequate EPA petition method validations have been conducted on wheat grain, straw, and forage for the two enforcement methods (D262416, Elmer Hayes, ACB/BEAD, 6/22/00; D267870, N. Dodd, 8/8/00). Both methods have been forwarded to FDA for publication in PAM, Vol. II (DP Barcode D271447, 4/2/02, N. Dodd; and memo dated 4/2/02 from N. Dodd to M. Wirtz). The validated LOQs for Method REM 138.01 are 0.05 ppm for wheat forage, hay, and straw, and 0.02 ppm for wheat grain, processed commodities, and aspirated grain fractions. The validated LOQ for Method REM 138.10 is 0.05 ppm for all wheat commodities.

860.1360 Multiresidue Methods

Multiresidue method testing data for cloquintocet-mexyl and its metabolite CGA-153433 in wheat grain were submitted in conjunction with the first petition request (D257181, 4/7/00, N. Dodd). Cloquintocet-mexyl and CGA-153433 were tested through the FDA multiresidue methods according to the decision tree and protocols in the Pesticide Analytical Manual, Volume I, Appendix II. Cloquintocet-mexyl was tested per Protocols C, D, and E; recovery was variable using protocol D, and the test substance was not recovered using Protocol E. CGA-153433 was tested per Protocols B and C; the compound was not recovered using Protocol B, and based on the results of Protocol C testing, no further testing was required for this compound. The submitted multiresidue methods data have been forwarded to FDA for review to determine sufficiency (D255566, N. Dodd, 5/12/99).

860.1380 Storage Stability

46012916.der.wpd
46012917.der.wpd

Deficiencies # 's 17a, 17b, and 17c from D257181:

In the 4/7/00 review, RAB3 concluded that additional storage stability data were needed as follows:

> 17a. Additional data are needed for Study 300/91 (MRID 44399210). Raw data, including residues (ppm) found and representative chromatograms (for standards, controls, freshly fortified samples, and stored samples) should be submitted. Storage containers should be described. The method used to analyze the storage stability samples should be submitted or identified by number as a submitted method.
>
> 17b. No storage stability data were submitted for forage. Storage stability data for forage are needed for the105-day storage interval for CGA-185072 and the 218-day storage interval for CGA-153433 in US residue samples. If the Canadian studies could be used (i.e., upgraded to acceptable), storage stability data for forage would be needed for the 434-day storage interval for CGA-185072 and CGA-153433 in the Canadian residue samples.
>
> 17c. Storage stability data are needed for CGA-153433 in wheat germ for 45 and 125 days.

Petitioner's Response to Deficiency 17a from D257181:

None.

Conclusion 17a:

Deficiency #17a has not been resolved. The petitioner must submit the following additional data to support the storage stability study (Study 300/91; MRID 44399210) for CGA-153433 in wheat grain: (1) raw data, including residues (ppm) found and representative chromatograms (for standards, controls, freshly fortified samples, and stored samples); (2) description of storage containers; and (3) submission or identification by number of the method used to analyze the storage stability samples.

Petitioner's Response to Deficiency 17b from D257181:

In response, Syngenta submitted a storage stability study (MRID 46012916) reflecting the stability of residues of cloquintocet-mexyl and CGA-153433 in wheat forage and hay during frozen storage.

MRID 46012916: Syngenta has submitted the results of a storage stability study of cloquintocet-mexyl and its metabolite CGA-153433 in/on wheat forage and hay. Untreated samples of wheat

forage and hay were fortified with cloquintocet-mexyl and its metabolite CGA-153433 at 1.00 ppm each. Fortified and unfortified samples of wheat forage and hay were extracted following 0, 14, 29, 61, 120, 180, 275, 359, 533, and 769 days of storage for cloquintocet-mexyl, and following 0, 90, 180, 359, 539, and 770 days of storage for metabolite CGA-153433. For cloquintocet-mexyl, extracts were stored for an additional 0-13 days until analysis. For metabolite CGA-153433, extracts were stored for an additional 1-13 days until analysis.

Samples of wheat forage and hay were analyzed for residues of cloquintocet-mexyl using HPLC/UV method REM 138.01 and were analyzed for residues of the metabolite CGA-153433 using a modified version of HPLC Method REM 138.10; the method was modified to use MS detection of CGA-153433 (instead of UV detection). The validated LOQ was 0.05 ppm for each analyte in wheat forage and hay. These methods are adequate for data collection based on acceptable method recoveries.

The submitted storage stability data indicate that residues of cloquintocet-mexyl are reasonably stable in/on wheat forage and hay for up to ~18 months. When compared to Day-0 recovery values, residues of cloquintocet-mexyl appear to decline in wheat forage and hay by ~45-50% after ~25 months of frozen storage.

The submitted storage stability data indicate that residues of metabolite CGA-153433 are reasonably stable in/on wheat forage and hay for up to ~25 months of frozen storage.

Conclusion 17b

Deficiency #17b is resolved by submission of a storage stability study (MRID 46012916) reflecting the stability of residues of cloquintocet-mexyl and CGA-153433 in wheat forage and hay during frozen storage. The submitted storage stability data indicate that residues of cloquintocet-mexyl are reasonably stable in/on wheat forage and hay for up to ~18 months. When compared to Day-0 recovery values, residues of cloquintocet-mexyl appear to decline in wheat forage and hay by ~45-50% after ~25 months of frozen storage. The submitted storage stability data indicate that residues of metabolite CGA-153433 are reasonably stable in/on wheat forage and hay for up to ~25 months of frozen storage.

Petitioner's Response to Deficiency 17c from D257181:

In response, Syngenta submitted a storage stability study (MRID 46012917) reflecting the stability of residues of CGA-153433 in wheat germ during frozen storage.

MRID 46012917: Syngenta has submitted the results of a storage stability study with metabolite CGA-153433 in wheat germ. Untreated samples of wheat germ were fortified with CGA-153433 at 1.00 ppm. Fortified and unfortified samples of wheat germ were extracted following 0, 33, 95, and 160 days of storage. With the exception of the 0-day sample, extracts were stored for an additional 2-5 days until analysis. The 0-day sample extract was stored for 33 days prior to analysis.

Samples of wheat germ were analyzed for residues of CGA-153433 using a modified version of HPLC Method REM 138.10; the method was modified to use MS detection of CGA-153433 (instead of UV detection). The reported LOQ was 0.05 ppm for wheat germ. The method was adequate for data collection based on acceptable method recoveries.

The results indicate that residues of the metabolite CGA-153433 are reasonably stable in wheat germ for up to 165 days (5.4 months) of frozen storage.

Conclusion 17c:

Deficiency #17c is resolved by submission of the storage stability study (MRID 46012917) for CGA-153433 in wheat germ. The study indicates that residues of the metabolite CGA-153433 are reasonably stable in wheat germ for up to 165 days (5.4 months) of frozen storage.

Discussion

Storage intervals and conditions of samples from studies reported in this document: All wheat samples were stored frozen (~-20 °C) prior to analysis. The maximum storage intervals of wheat samples from harvest to analysis for cloquintocet-mexyl were 187 days (6.1 months) for forage, 134 days (4.4 months) for hay, 148 days (4.9 months) for straw, and 141 days (4.6 months) for grain. The maximum storage intervals of crop samples from harvest to analysis for CGA-153433 were 253 days (8.3 months) for forage, 231 days (7.6 months) for hay, 308 days (10.1 months) for straw, and 222 days (7.3 months) for grain.

The maximum storage intervals of processing study samples from harvest of wheat grain to analysis for cloquintocet-mexyl were 147 days (4.8 months) for wheat grain, 140 days (4.6 months) for aspirated grain fractions, and 153 days (5.0 months) for wheat processed commodities. The maximum storage intervals of processing study samples from harvest of grain to analysis for CGA-153433 were 159 days (5.2 months) for wheat grain, 161 days (5.3 months) for aspirated grain fractions and wheat processed fractions other than germ, and 158 days (5.2 months) for germ.

Barley grain, hay, and straw were stored 36-149 days. Barley processed commodities (pearled barley, flour, and bran) were stored 4.3-14.9 months.

Available storage stability data. The available storage stability data indicate that residues of cloquintocet-mexyl (parent) are reasonably stable in/on wheat forage and hay for up to ~18 months of frozen storage and residues of metabolite CGA-153433 are reasonably stable in/on wheat forage and hay for up to ~25 months of frozen storage (MRID 46012916). Residues of cloquintocet-mexyl (parent) are stable in/on wheat straw stored at -18 °C for 6 months (MRID 44399209). Residues of CGA-153433 are stable in/on wheat straw at -18 °C for 380 days (MRID 44399207). Residues of cloquintocet-mexyl (parent) are stable in/on wheat grain stored at -18 °C for 178 days (approx 6 months) (MRID 44399208). Pending receipt of additional information for Study 300/91 (MRID 44399210), HED tentatively concluded that CGA-153433 is stable in wheat grain stored at -20 °C for up to 727 days.

The available storage stability data indicate that residues of the metabolite CGA-153433 are reasonably stable in wheat germ for up to 165 days (5.4 months) of frozen storage (MRID 46012917).

Storage stability data for aspirated grain fractions or wheat processed commodities other than wheat germ have not been submitted or requested. The storage time between processing and analysis was ≤25 days for CGA-185072; storage stability data are not needed for CGA-185072 in processed commodities since they were analyzed within 30 days of their production (OPPTS 860.1520). The storage times between processing and analysis for CGA-153433 were 51 days for aspirated grain fractions, 45 and 125 days for germ, 45 days for bran, 42 days for middlings and shorts, and 37 days for low grade flour and patent flour. Storage stability data for CGA-153433 in aspirated grain fractions are not needed since this is an early season use and residues are not expected to occur in aspirated grain fractions. Storage stability data are not needed for bran, flour, middlings, and shorts since these matrices are similar to wheat grain and can be covered by the storage stability data on grain.

Storage stability data for barley processed commodities (pearled barley, flour, and bran) are not needed since these matrices are similar to barley grain and can be covered by the storage stability data on grain.

Conclusions. The available storage stability data are adequate to support the storage conditions and storage intervals for all wheat and barley commodities except grain. The petitioner must submit the following additional data to support the storage stability study (Study 300/91; MRID 44399210) for CGA-153433 in wheat grain: (1) raw data, including residues (ppm) found and representative chromatograms (for standards, controls, freshly fortified samples, and stored samples); (2) description of storage containers; and (3) submission or identification by number of the method used to analyze the storage stability samples.

860.1400 Water, Fish, and Irrigated Crops

There are no proposed uses that are relevant to this guideline topic.

860.1460 Food Handling

There are no proposed uses that are relevant to this guideline topic.

860.1480 Meat, Milk, Poultry, and Eggs

No ruminant feeding study has been submitted. In the goat metabolism study (MRID's 44387458 and 44387460), a lactating goat was dosed with (3-^{14}C)quinoline-labeled cloquintocet-mexyl for ten consecutive days at a dose level of 5 ppm. Maximum total radioactive residues found at the 5 ppm dose level were 0.084 ppm in milk, 0.003 ppm in muscle, 0.001 ppm in fat, 0.024 ppm in kidney, and 0.010 ppm in liver.

No poultry feeding study was submitted. In the poultry metabolism study (MRID's 44387459 and 44387461), three laying hens were dosed with (3-^{14}C)quinoline-labeled cloquintocet-mexyl for fourteen consecutive days at a dose level of 5 ppm. Maximum total radioactive residues found in muscle, fat, liver, and eggs at the 5 ppm dose were nondetectable (<0.001 ppm) in muscle, nondetectable (<0.002 ppm) in fat, 0.01 ppm in liver, and 0.006 ppm in eggs.

In the 4/7/00 review, RAB3 concluded that because of the low residue levels found in milk, egg, and tissues in the goat and hen metabolism studies, livestock feeding studies were not needed. Tolerances for livestock commodities were not required to support the proposed use of cloquintocet-mexyl as a safener with clodinafop-propargyl; this use fell under 40 CFR §180.6(a)(3) since no secondary residues were expected to occur in eggs, milk, and the meat, fat, and meat byproducts of cattle, goats, hogs, horses, poultry, and sheep.

Because wheat tolerances have been revised and use on barley has been proposed, the maximum theoretical dietary burden of cloquintocet-mexyl to livestock was recalculated. The maximum theoretical dietary burden of cloquintocet-mexyl to livestock from uses on wheat and barley is presented in Table 5 below.

Table 5. Calculation of Maximum Dietary Burdens of Cloquintocet-mexyl to Livestock.				
Feedstuff	% Dry Matter[1]	% Diet[1]	Estimated Tolerance (ppm)	Dietary Contribution (ppm)[2]
Beef Cattle				
Wheat, grain	89	50	0.1	0.06
Wheat, forage	25	25	0.1	0.10
TOTAL BURDEN	--	75[3]	--	0.16
Dairy Cattle				
Wheat, grain	89	25	0.1	0.03
Wheat, forage	25	60	0.1	0.24
TOTAL BURDEN	--	85[3]	--	0.27
Poultry				
Wheat, grain	--	80	0.1	0.08
TOTAL BURDEN	--	80[3]	--	0.08
Swine				
Wheat, grain	--	80	0.1	0.08
TOTAL BURDEN		80[3]	--	0.08

[1] Table 1 (OPPTS Guideline 860.1000).

[2] Contribution = ([tolerance /% DM] x % diet) for beef and dairy cattle; contribution = (tolerance x % diet) for poultry and swine.

[3] The remainder of the diet will be composed of feedstuff derived from crops that do not have cloquintocet-mexyl uses/tolerances proposed.

Residue levels in livestock commodities in the metabolism studies conducted at 5 ppm are extracted to1X levels (calculated in Table 5 above) in the following table:

Table 6. Total Radioactive Residues (TRR) in Goat and Poultry Metabolism Studies Conducted at a 5.0 ppm Dosing Level and Extrapolated to 1X (0.16 ppm for Beef Cattle, 0.27 ppm for Dairy Cattle, 0.08 ppm for Swine, and 0.08 ppm for Poultry)		
Substrates	TRR from 5 ppm Dosing Level (ppm)	TRR Extrapolated to1X (ppm)
Beef Cattle		
muscle (tenderloin)	0.003	0.000096
fat (subcutaneous)	0.001	0.000032
kidney	0.024	0.00077
liver	0.010	0.00032
Dairy Cattle		
milk (maximum)	0.084	0.0045
muscle (tenderloin)	0.003	0.00016
fat (subcutaneous)	0.001	0.000054
kidney	0.024	0.0013
liver	0.010	0.00054
Swine		
muscle (tenderloin)	0.003	0.000048
fat (subcutaneous)	0.001	0.000016
kidney	0.024	0.00038
liver	0.010	0.00016
Poultry		
muscle	ND (<0.001)	0.000016
fat	ND (<0.002)	0.000032
liver	0.01	0.00016
eggs	0.006[1]	0.000096

[1] Maximum residue, found in egg from Hen 108F at 312 hrs (30% of 0.0040 ppm in yolk + 70% of 0.0071 ppm in white).

Conclusions. Because of the low levels of total radioactive residues found in livestock commodities in the ruminant and poultry metabolism studies and the corresponding low radioactive residues calculated for the 1X feeding levels, ruminant and poultry feeding studies are not needed and tolerances on livestock commodities are not needed. The uses on wheat and barley fall under 40 CFR §180.6(a)(3) since no secondary residues are expected to occur in livestock commodities.

860.1500 Crop Field Trials

Wheat

46012904.der.wpd
46012905.der.wpd
46012918.der.wpd
46203206.der.wpd

Deficiency #18 (a, b, c, d) from D257181:

In the previous review for cloquintocet-mexyl (D257181, N. Dodd, 4/7/00), the US field trial data (six field trials on spring wheat in MRID 44755303, an interim report) were not adequate for the following reasons:

18a. Adequate geographic representation was not provided. To support a 30-day PHI in forage (as currently on the label), an additional 14 field trials are required, in Region 2 (1 trial), Region 4 (1 trial), Region 5 (3 trials), Region 6 (1 trial), Region 7 (1 trial), Region 8 (6 trials), and Region 11 (1 trial). Spring wheat (including hard red spring, duram and white spring varieties) and winter wheat (including hard red winter, soft red winter, and white winter varieties) should be included. (If the petitioner could not analyze the spring wheat straw samples (included in the interim report) for residues of CGA-153433 and support the reanalyses with storage stability data, then additional crop field trial data would be required.) Each study should include DSV Adjuvant or similar adjuvant. Raw data and representative chromatograms of standards, controls, fortified samples, and treated samples should be included. Storage information including types of storage containers and dates of extraction (as well as dates of storage and analysis) should be included.

18b. Only spring wheat was used in the US and Canadian studies. Winter wheat should also be included in the residue studies.

18c. Forage was sampled at the proposed preharvest interval (PHI) of 7 days in only one US study and three Canadian studies.

18d. Based on the available residue data, the petitioner should submit a revised Section F which proposes tolerances of 0.10 ppm for the combined residues of cloquintocet-mexyl and its metabolite 5-chloro-8-quinolinoxyacetic acid on wheat grain, forage, hay, and straw.

These levels were obtained by adding the limits of quantitation of CGA-185072 and CGA-153433.

Deficiency #19 (a, b, c, d, and e) from D257181:

In the previous review for cloquintocet-mexyl (D257181, N. Dodd, 4/7/00), the Canadian field trial data were not adequate for the following reasons:

19a. Grain, forage, hay, and straw were not analyzed in each of the wheat field trial residue studies. Of the 15 Canadian studies, only grain and straw were analyzed in most studies (i.e., in twelve studies for CGA-185072 and nine studies for CGA-153433), and only forage was analyzed (for both CGA-185072 and CGA-153433) in three studies. Hay was not analyzed.

19b. PHI's did not reflect the proposed use. PHI's for grain and straw in the Canadian studies ranged from 55-105 days (with all but two studies with PHI's above 60 days) whereas the proposed PHI for grain and straw is 60 days.

19c. Extraction dates were not provided for studies in MRIDs 44399217-44399227 and 44399231.

19d. Storage containers were not described.

19e. Raw data and representative chromatograms of standards, controls, fortified samples, and treated samples were not submitted.

Petitioner's Response to Deficiencies #'s 18a and 18b:

Syngenta has now submitted the final report for the U.S. crop field trial submission reviewed previously (MRID 46012904). Additional field trial data have been submitted in MRIDs 46012905, 46012918 and 46203206).

Table 7. Summary of Residues from the Crop Field Trials with Cloquintocet-Mexyl.

Commodity	Total Applic. Rate, lb ai/A [g ai/A]	PHI (days)	Analyte	Residue Levels (ppm)						
				n	Min.	Max.	HAFT[1]	Median	Mean	Std. Dev.
Wheat (proposed use = 0.016 lb safener/A total application rate, 30-day PHI for forage and hay, 60-day PHI for grain and straw)										
Spring wheat (MRID 46012904)										
Wheat, forage	0.016 [7.1]	29-32	CGA-185072	12	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			CGA-153433	12	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			Total	12	<0.10	<0.10	<0.10	<0.10	<0.10	0.0
Wheat, hay	0.016 [7.1]	29-47	CGA-185072	12	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			CGA-153433	12	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			Total	12	<0.10	<0.10	<0.10	<0.10	<0.10	0.0
Wheat, straw	0.016 [7.1]	57-61	CGA-185072	12	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			CGA-153433	12	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			Total	12	<0.10	<0.10	<0.10	<0.10	<0.10	0.0
Wheat, grain	0.016 [7.1]	57-61	CGA-185072	12	<0.02	<0.02	<0.02	<0.02	<0.02	0.0
			CGA-153433	12	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			Total	12	<0.07	<0.07	<0.07	<0.07	<0.07	0.0
Winter wheat treated in the spring (MRID 46012904)										
Wheat, forage	0.016 [7.1]	29-32	CGA-185072	12	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			CGA-153433	12	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			Total	12	<0.10	<0.10	<0.10	<0.10	<0.10	0.0
Wheat, hay	0.016 [7.1]	29-32	CGA-185072	12	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			CGA-153433	12	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			Total	12	<0.10	<0.10	<0.10	<0.10	<0.10	0.0
Wheat, straw	0.016 [2] [7.1]	58-69	CGA-185072	12	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			CGA-153433	12	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			Total	12	<0.10	<0.10	<0.10	<0.10	<0.10	0.0
Wheat, grain	0.016 [2] [7.1]	58-69	CGA-185072	12	<0.02	<0.02	<0.02	<0.02	<0.02	0.0
			CGA-153433	12	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			Total	12	<0.07	<0.07	<0.07	<0.07	<0.07	0.0
Winter wheat treated in the fall (MRID 46012904) *										
Wheat, forage	0.016 [7.1]	27-33	CGA-185072	16	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			CGA-153433	16	<0.05	0.07	0.07	<0.05	<0.05	0.0
			Total	16	<0.10	<0.12	<0.12	<0.10	<0.10	0.0

Table 7. Summary of Residues from the Crop Field Trials with Cloquintocet-Mexyl.

Commodity	Total Applic. Rate, lb ai/A [g ai/A]	PHI (days)	Analyte	Residue Levels (ppm)						
				n	Min.	Max.	HAFT[1]	Median	Mean	Std. Dev.
Wheat, hay	0.016 [7.1]	27-33	CGA-185072	16	<0.05	0.06	0.06	<0.05	<0.05	0.0
			CGA-153433	16	<0.05	0.13	0.13	<0.05	<0.05	0.0
			Total	16	<0.10	0.19	0.19	<0.10	<0.10	0.0
Wheat, straw	0.016 [7.1]	58-63	CGA-185072	16	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			CGA-153433	16	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			Total	16	<0.10	<0.10	<0.10	<0.10	<0.10	0.0
Wheat, grain	0.016 [7.1]	58-63	CGA-185072	16	<0.02	<0.02	<0.02	<0.02	<0.02	0.0
			CGA-153433	16	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			Total	16	<0.07	<0.07	<0.07	<0.07	<0.07	0.0
Winter wheat treated in the spring (MRID 46012905)										
Wheat, forage	0.016 [7.1]	29-30	CGA-185072	16	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			CGA-153433	16	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			Total	16	<0.10	<0.10	<0.10	<0.10	<0.10	0.0
Wheat, hay	0.016 [7.1]	29-30	CGA-185072	16	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			CGA-153433	16	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			Total	16	<0.10	<0.10	<0.10	<0.10	<0.10	0.0
Spring and winter wheat grown in the Pacific Northwest (MRID 46012918)										
Wheat, forage	0.016 [7.1]	29-30	CGA-185072	6	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			CGA-153433	6	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			Total	6	<0.10	<0.10	<0.10	<0.10	<0.10	0.0
Wheat, hay	0.016 [7.1]	29-30	CGA-185072	6	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			CGA-153433	6	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			Total	6	<0.10	<0.10	<0.10	<0.10	<0.10	0.0
Wheat, straw	0.016 [7.1]	56-60	CGA-185072	8	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			CGA-153433	8	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			Total	8	<0.10	<0.10	<0.10	<0.10	<0.10	0.0
Wheat, grain	0.016 [7.1]	56-60	CGA-185072	8	<0.02	<0.02	<0.02	<0.02	<0.02	0.0
			CGA-153433	8	<0.05	<0.05	<0.05	<0.05	<0.05	0.0
			Total	8	<0.07	<0.07	<0.07	<0.07	<0.07	0.0

[1] HAFT = Highest Average Field Trial.

[2] In one trial, wheat plants inadvertently received both an early season and late season application, yielding an application rate of 0.032 lb ai/A (14.2 g ai/A).

* These studies on winter wheat treated in the fall should be disregarded because the labels will prohibit use on wheat in the fall.

MRID 46012904: Syngenta has submitted the final report for field trial data on wheat forage, hay, straw, and grain. An interim report of these wheat field trial data was reviewed in the 4/7/00 review; that submission included only the results from spring wheat crop field trials and did not include the results of analyses of spring wheat straw samples for CGA-153433. We note that the discussion of results below includes the results of the spring wheat field trials that were reported in the 4/7/00 review.

A total of 20 wheat field trials (6 spring wheat trials and 14 winter wheat trials) were conducted during the 1998-1999 growing seasons. Spring wheat field trials were conducted in Regions 5 (MN and ND; 2 trials) and 7 (MT, ND, and SD; 4 trials). Winter wheat field trials were conducted in Regions 2 (NC; 1 trial), 4 (AR; 1 trial), 5 (IL, KS, and MO; 3 trials), 6 (OK; 1 trial), 8 (CO, KS, NM, OK, and TX; 6 trials), and 11 (WA; 1 trial). In addition, one trial was conducted in Region 5 (NE) close enough to the border with Region 7 to support geographic representation requirements for Region 7.

The winter wheat field trials were conducted with two types of early season application: application in the fall or application in the spring. Of the 14 winter wheat trials, a total of 8 trials were conducted reflecting application in the fall, in Regions 2 (NC; 1 trial), 4 (AR; 1 trial), 5 (KS; 1 trial), 6 (OK; 1 trial), and 8 (KS, OK, and TX; 4 trials). The remaining 6 trials were conducted reflecting early season application in the spring, in Regions 5 (IL and MO; 2 trials), 7 (NE; 1 trial), 8 (CO and NM; 2 trials), and 11 (WA; 1 trial). The Discover®/Discover® NG Herbicide labels prohibit application to winter wheat in the fall. The petitioner has stated that they will not be supporting application of Discover®/Discover® NG Herbicide to winter wheat in the fall; application to winter wheat will be restricted to a spring postemergence foliar application. Because application to winter wheat in the fall will be prohibited, the data on winter wheat treated in the fall will be disregarded. HED notes that harvest of winter wheat hay 0 or 30 days following application in the fall is not typical agronomic practice; in general, if herbicides are applied to winter wheat in the fall, hay would not typically be harvested until the spring.

At each field trial site, separate plots were treated with a single postemergence foliar application of an EC formulation containing 0.5 lb/gal of the safener cloquintocet-mexyl and 2 lb ai/gal of the herbicide clodinafop-propargyl; the various treatments are described below. Applications to most plots were made using ground equipment in 5-20 gal/A of water with an adjuvant added to the spray mixture. At three locations (one spring wheat and two winter wheat), applications were made using a concentrated spray volume (2 gal/A) to simulate aerial application.

Table 8. Description of Application of Cloquintocet-mexyl to Wheat (MRID 26012904)		
Treatment Plot	**Description**	**Wheat RAC Sampled/Analyzed**
1	Control; none applied.	Forage, hay, and mature straw and grain
2	Single postemergence foliar application in the fall or spring of the 0.5 lb safener/gal EC formulation made to wheat early in the season at Feekes Growth Stage 2 at 0.016 lb safener/A (7.1 g safener/A); 1x the proposed maximum seasonal rate.	Forage and hay
3	Single postemergence foliar application in the spring of 0.5 lb safener/gal EC formulation made to wheat approximately 60 days prior to harvest of mature wheat, at 0.016 lb safener/A (7.1 g safener/A); 1x the proposed maximum seasonal rate.	Mature straw and grain

Samples of wheat forage were collected at a 0- and a 27- to 33-day PHI; samples of wheat hay were collected at a 27- to 47-day PHI from treatment plot 2; and samples of wheat straw and grain were collected at maturity (57- to 63-day PHI) from treatment plot 3. To demonstrate residue decline, additional samples of wheat forage and hay were collected at posttreatment intervals of 0, 7, ~14, ~21, ~30, and 37 days from treatment plot 2, and wheat straw and grain were collected at posttreatment intervals of ~45, ~52, ~60, and ~66 days from treatment plot 3 from one spring wheat trial (ND) and two winter wheat trials (KS and OK). The results of the crop field trials are presented in Table 7 above.

Residue decline data show that residues of cloquintocet-mexyl and CGA-153433 decrease in/on wheat forage and hay with increasing sampling intervals, with the maximum residues occurring at the 0-day sampling interval. Residues of cloquintocet-mexyl were <0.05 ppm and <0.02 ppm in/on wheat straw and grain, respectively, and residues of CGA-153433 were <0.05 ppm at all sampling intervals (45-67 days posttreatment) in the decline studies.

Samples of wheat forage, hay, grain, and straw were analyzed for residues of cloquintocet-mexyl using HPLC/UV Method REM 138.01; the validated LOQs were 0.05 ppm for wheat forage, hay, and straw, and 0.02 ppm for wheat grain. Samples were analyzed for residues of the metabolite CGA-153433 using HPLC/UV Method REM 138.10; the validated LOQ was 0.05 ppm for wheat forage, hay, straw, and grain. These methods are adequate for data collection based on acceptable method recoveries. When the results of the spring wheat field trials were submitted previously (under MRID 44755303; see 4/7/00 review), the petitioner reported that straw samples could not be analyzed for residues of CGA-153433 "using current methodology." The current submission includes results for straw samples, which appear to have been analyzed for CGA-153433 after the previous submission. The petitioner did not provide any discussion of this issue (i.e., did not explain whether changes to the method were required to allow analysis of straw samples) and did not provide any raw data for the analyses of spring wheat straw samples for CGA-153433.

The maximum storage intervals of crop samples from harvest to analysis for cloquintocet-mexyl were 6.1 months for forage, 4.4 months for hay, 4.9 months for straw, and 4.6 months for grain. The maximum storage intervals of crop samples from harvest to analysis for CGA-153433 were 8.3 months for forage, 7.6 months for hay, 10.1 months for straw, and 7.3 months for grain. The available storage stability data support the storage conditions and intervals of samples from the submitted wheat field trials.

46012905: Syngenta submitted data from additional field trials for winter wheat forage and hay reflecting treatment in the spring. The petitioner conducted a total of eight winter wheat field trials during the 2001 growing season in Regions 2 (NC; 1 trial), 4 (AR; 1 trial), 5 or 8 (KS; 1 trial, on the border between Regions 5 and 8), 6 (OK; 1 trial), and 8 (CO, KS, OK, and TX; 4 trials).

At each field trial site, winter wheat was treated with a single postemergence foliar application of an EC formulation containing 0.5 lb/gal of the safener cloquintocet-mexyl and 2 lb ai/gal of the herbicide clodinafop-propargyl. Application was made to winter wheat in the spring, at Feekes Growth Stage 2, at a rate of 0.016 lb safener/A (7.1 g safener/A); 1x the proposed maximum seasonal rate. Applications were made using ground equipment in 10.6-15.1 gal/A of water with an adjuvant added to the spray mixture. Samples of wheat forage and hay were collected at a 29- to 30-day PHI. In one field trial, additional samples of wheat forage and hay were collected at 0-, 7-, 14-, 21-, 30-, and 37-day PHIs to demonstrate residue decline.

The results of the crop field trials are presented in Table 7 above. Residue decline data show that residues of cloquintocet-mexyl and CGA-153433 decrease in/on wheat forage and hay with increasing sampling intervals, with quantifiable residues only occurring at the 0-day sampling interval.

Samples of wheat forage and hay were analyzed for residues of cloquintocet-mexyl using HPLC/UV method REM 138.01 and were analyzed for residues of the metabolite CGA-153433 using a modified version of HPLC Method REM 138.10; the method was modified to use MS detection of CGA-153433 (instead of UV detection). The validated LOQ was 0.05 ppm for each analyte in wheat forage and hay. These methods are adequate for data collection based on acceptable method recoveries. The maximum storage intervals of crop samples from harvest to analysis for cloquintocet-mexyl were 3.4 months for wheat forage and 3.8 months for wheat hay. The maximum storage intervals of crop samples from harvest to analysis for CGA-153433 were 3.3 months for wheat forage and 3.6 months for wheat hay. The available storage stability data support the storage conditions and intervals of samples from the submitted wheat field trials.

46012918: Syngenta has submitted field trial data on wheat forage, hay, straw, and grain from four wheat field trials (three spring wheat and one winter wheat) during the 2000 growing season in Region 11 (ID and WA). The petitioner intended the submitted field trials to represent the wheat-growing areas of the Pacific Northwest area of the U.S.

At each field trial site, separate plots were treated with a single postemergence foliar application of an EC formulation containing 0.5 lb/gal of the safener cloquintocet-mexyl and 2 lb ai/gal of the

herbicide clodinafop-propargyl; the various treatments are described below. Applications at all plots were made using ground equipment in 10-18 gal/A of water with an adjuvant added to the spray mixture.

Table 9. Description of Application of Cloquintocet-mexyl to Wheat (MRID 26012905)		
Treatment Plot	**Description**	**Wheat RAC Sampled/Analyzed**
1	Control; none applied.	Forage, hay, and mature straw and grain
2	Single postemergence foliar application of the 0.5 lb safener/gal EC formulation made to wheat early in the season at Feekes Growth Stage 2 at 0.016 lb safener/A (7.1 g safener/A); 1x the proposed maximum seasonal rate.	Forage and hay
3	Single postemergence foliar application of 0.5 lb safener/gal EC formulation made to wheat approximately 60 days prior to harvest of mature wheat, at 0.016 lb safener/A (7.1 g safener/A); 1x the proposed maximum seasonal rate.	Mature straw and grain

Samples of wheat forage were collected at a 0- and a 29- to 30-day PHI; samples of wheat hay were collected at a 29- to 30-day PHI from treatment plot 2; and samples of wheat straw and grain were collected at maturity (56- to 60-day PHI) from treatment plot 3.

The results of the crop field trials are presented in Table 7 above. No residue decline studies were conducted with these field trials. However, residue decline studies included in the other wheat field trial submissions indicated that residues of cloquintocet-mexyl and CGA-153433 do not increase in wheat commodities with increasing sampling intervals.

Samples of wheat RACs were analyzed for residues of cloquintocet-mexyl using HPLC/UV method REM 138.01; the validated LOQs were 0.05 ppm for wheat forage, hay, and straw, and 0.02 ppm for wheat grain. Samples were analyzed for residues of the metabolite CGA-153433 using a modified version of HPLC Method REM 138.10; the method was modified to use MS detection of CGA-153433 (instead of UV detection). The validated LOQ was 0.05 ppm for wheat forage, hay, straw, and grain. These methods are adequate for data collection based on acceptable method recoveries. The maximum storage intervals of crop samples from harvest to analysis for cloquintocet-mexyl were 2.9 months for wheat forage, 2.2 months for wheat hay, 2.0 months for wheat straw, and 2.1 months for wheat grain. The maximum storage intervals of crop samples from harvest to analysis for metabolite CGA-153433 were 3.2 months for wheat forage, 2.4 months for wheat hay, 2.2 months for wheat straw, and 2.2 months for wheat grain. The available storage stability data support the storage conditions and intervals of samples from the submitted wheat field trials.

MRID 46203206: Syngenta Crop Protection has submitted field trial data for cloquintocet-mexyl (CGA-185072) on wheat in Canada. Twenty trials were conducted in Canada encompassing Regions 5 (2, Manitoba), 7 (4, Alberta; 3, Saskatchewan), 7A (1, Alberta), and 14 (4, Alberta; 3, Manitoba; 3, Saskatchewan) during the 2003 growing season.

Cloquintocet-mexyl is a safener that is applied in the herbicide formulation NOA-407855. At each field trial location, treatment consisted of a single broadcast spray application of NOA-407855 formulation 100EC Lead Variant (A-12303C). At three sites, two additional plots were treated with a single broadcast spray application of other NOA-407855 formulations. On one of the additional plots the NOA-407855 formulation was100EC Alternate Variant (A-12303D), and on the other it was NOA-407855 formulation 120EC Aromatic 200 (A-12413B). All of these formulations contain cloquintocet-mexyl as a safener. All treatments had an application of cloquintocet-mexyl at 0.016 lb a.i./A (0.018 kg a.i./ha). One of the following adjuvant activators was added to the spray mixture for all applications: A12127, A12127S, or MERGE. The single application was applied up to the crop growth stage BBCH 23. Excluding the last two treatments in the two decline trials, pre-harvest intervals (PHIs) for forage, hay, grain and straw were 4-25, 22-50, 58-98 and 58-98 days, respectively.

Residues of metabolite CGA-153433 were quantified using Analytical Method REM 199.02. The method converts cloquintocet-mexyl to this acid metabolite. Quantification of residues was based on LC/MS/MS (liquid chromatography coupled to tandem mass spectrometry). Satisfactory method performance in detecting CGA-153433 was demonstrated by concurrent recoveries. Freezer storage intervals ranged from 36-149 days after harvest. Residues of the cloquintocet-mexyl have been shown to be stable in all matrices for the duration of storage that occurred during the conduct of this study. The results from these trials show that maximum CGA-153433 residues were 0.114 ppm in forage, < 0.02 ppm (the LOQ) in hay and straw, and <0.01 ppm (the LOQ) in grain. Residue decline data show that residues of CGA-153433 on forage decrease with increasing PHIs. Since there were no quantifiable residues on hay, grain, and straw at any PHIs, the decline studies provided no evidence that cloquintocet-mexyl residues increase with increasing PHIs on those commodities.

Table 10. Summary of Residues from the Crop Field Trials with Cloquintocet-Mexyl conducted in Canada (MRID 46302206)										
Commodity	Total Applic. Rate, lb ai/A [g ai/A]	PHI (days)	Analyte [2]	Residue Levels (ppm) [3]						
				n	Min.	Max.	HAFT[1]	Median	Mean	Std. Dev.
Wheat (proposed use = 0.016 lb safener/A total application rate, 30-day PHI for forage and hay, 60-day PHI for grain and straw)										
Wheat (MRID 46203206)										
Wheat, forage	0.016 [7.1]	22-31	CGA-153433	8	<0.02	<0.02	<0.02	<0.02	<0.02	0.0
Wheat, hay	0.016 [7.1]	28-35	CGA-153433	29	<0.02	<0.02	<0.02	<0.02	<0.02	0.0
Wheat, straw	0.016 [7.1]	58-62	CGA-153433	23	<0.02	<0.02	<0.02	<0.02	<0.02	0.0
Wheat, grain	[7.1]	58-62	CGA-153433	23	<0.01	<0.01	<0.01	<0.01	<0.01	0.0

[1] HAFT = Highest Average Field Trial.

[2] The method (REM 199.02) determines parent and the metabolite CGA-153433 as CGA-153433.

[3] All values are reported as <LOQ in the table above. The LOD is ½ LOQ. Values were also <LOD in most cases.

Conclusions:

Deficiency #18a has not been resolved. The wheat field trials are not adequate in number or geographic representation. A total of 20 wheat field trials (6 spring wheat trials and 14 winter wheat trials) were conducted during the 1998-1999 growing seasons. Spring wheat field trials were conducted in Regions 5 (MN and ND; 2 trials) and 7 (MT, ND, and SD; 4 trials). Winter wheat field trials were conducted in Regions 2 (NC; 1 trial), 4 (AR; 1 trial), 5 (IL, KS, and MO; 3 trials), 6 (OK; 1 trial), 8 (CO, KS, NM, OK, and TX; 6 trials), and 11 (WA; 1 trial). In addition, one trial was conducted in Region 5 (NE) close enough to the border with Region 7 to support geographic representation requirements for Region 7. The winter wheat field trials were conducted with two types of early season application: application in the fall or application in the spring. Of the 14 winter wheat trials, a total of 8 trials were conducted reflecting application in the fall, in Regions 2 (NC; 1 trial), 4 (AR; 1 trial), 5 (KS; 1 trial), 6 (OK; 1 trial), and 8 (KS, OK, and TX; 4 trials). The remaining 6 trials were conducted reflecting early season application in the spring, in Regions 5 (IL and MO; 2 trials), 7 (NE; 1 trial), 8 (CO and NM; 2 trials), and 11 (WA; 1 trial). The petitioner has stated that they will not be supporting application of Discover®/Discover® NG Herbicide to winter wheat in the fall; application to winter wheat will be restricted to a spring postemergence foliar application. Because application to winter wheat in the fall is restricted, the data can be disregarded for Discover®/Discover® NG Herbicide; however, not using these studies as part of the database means that geographic representation of the residue trials is not adequate.

Because the data for the winter wheat treated in the spring will not be used, the number and geographic representation for the wheat field trials are not adequate. For Discover®/Discover® NG

Herbicide, an additional 8 field trials are needed for adequate geographic representation as follows: 1 trial in Region 2, 1 trial in Region 4, 1 trial in Region 5, 1 trial in Region 6, and 4 trials in Region 8. For Axial™ Herbicide, the petitioner should add the restriction "Do not apply to winter wheat in the fall" to the label; this restriction is already on the Discover®/Discover® NG Herbicide labels.

Alternatively, the winter wheat data could be used but the higher tolerances then needed on wheat commodities would mean that a ruminant feeding study would be needed or a livestock method for meat, fat, and milk including radiovalidation data and an independent laboratory validation would be needed. A validation of the livestock method by EPA's ACB/BEAD would also be needed. If a validated livestock enforcement method could be made available, tolerances for ruminant commodities, pending ChemSAC approval, could be set at the limit of quantitation of the method.

Deficiency #18b has been resolved since data on both spring wheat and winter wheat were submitted.

Deficiencies #'s18c and 18d have been previously resolved by submission of a revised Section B (proposing a 30-day PHI for forage) and a revised Section F (proposing the requested tolerance).

Deficiencies 19 (a-e) refer to studies conducted in Canada which have not been upgraded. HED did not recommend that the petitioner attempt to upgrade these studies.

The submitted wheat field trials are adequate for a conditional registration. Samples of wheat forage, hay, grain, and straw were analyzed. Most of the wheat samples were analyzed by the HPLC/UV enforcement methods REM 138.01 (for parent) and REM 183.10 (for the metabolite CGA-153433). The validated LOQs for Method REM 138.01 are 0.05 ppm for wheat forage, hay, and straw, and 0.02 ppm for wheat grain, processed commodities, and aspirated grain fractions. The validated LOQ for Method REM 138.10 is 0.05 ppm for all wheat commodities. Residues in spring wheat and winter wheat planted in the spring will not exceed 0.1 ppm in wheat grain, forage, hay, and straw.

Barley

46203204.der.wpd
46203205.der.wpd

Table 11. Summary of Residue Data from Barley Field Trials with Cloquintocet-mexyl, as Safener in Herbicide NOA-407855 (MRIDs 46203204 and 46203205)

Commodity	Total Applic. Rate, (lb a.i./A)	PHI (days)	Residue Levels of CGA-153433 (ppm)[1]						
			n	Min.	Max.	HAFT[2]	Median	Mean	Std. Dev.
CGA-153433 (metabolite of safener Cloquintocet-mexyl)									
Barley hay	0.016	26-35	48	<LOQ[3]	0.048	0.029	<LOQ	<LOQ	N/A[4]
Barley straw	0.016	57-66	44	<LOQ	0.050	0.036	<LOQ	<LOQ	N/A
Barley grain	0.016	57-66	44	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	N/A

[1] The method determines parent cloquintocet-mexyl and its metabolite CGA-153433 as CGA-153433.
[2] HAFT = Highest Average Field Trial.
[3] LOQ =0.01 ppm for grain and 0.02 ppm for hay and straw.
[4] N/A = not applicable.

MRID 46203204: Syngenta Crop Protection, Inc. has submitted field trial data for the safener cloquintocet-mexyl (CGA-185072) on barley. Residues of the safener metabolite CGA-153433 were measured on barley as part of a field trial for the new emulsifiable concentrate herbicide. A total of 12 field trials were conducted in Regions 2 (VA), 5 (ND, MN, WI), 7 (SD, ND, MT [2]), 9 (CO), 10 (CA), and 11 (WA, ID) during the 2002 growing season. Barley was grown under normal agricultural conditions at each field trial location. The number and locations of field trials are in accordance with OPPTS Guideline 860.1500.

The herbicide formulation was diluted with water for a targeted treatment rate of 0.016 lb cloquintocet-mexyl/A (0.018 kg/ha; 1X) for hay, straw, and grain. Treatment rates of 3X and 5X were also applied for straw and grain. All treatments were made by one-time post-foliar broadcast spray using 2-20 gallons/acre. Hay samples were cut approximately 30 days after treatment and straw and grain were harvested 60 days after treatment. Additional samples from the ID trial were used to generate processed grain fractions.

Syngenta Analytical Method 117-01 was used for determination of residues of CGA-153433 in barley samples. During the acid refluxing of the sample, cloquintocet-mexyl is oxidized to CGA-153433. The LOD was 0.005 ppm. The LOQ was 0.01 ppm for grain and processed grain fractions, and 0.02 ppm for hay and straw. The quantitation of residues was based on HPLC/MS/MS peak area comparison with calibration standard solutions. Concurrent recovery samples were prepared at the LOQ level up to 5 ppm for each RAC sample. Recoveries ranged from 81-113% (n=107) for CGA-153433. Storage stability data for CGA-153433 on barley RAC were not included although limited data on wheat were available. Barley commodities (grain, hay, and straw) were stored 36-149 days.

Minimal concentrations of the safener metabolite CGA-153433 were found in only one hay sample (0.048 ppm) and in three straw samples (0021-0.050 ppm); all remaining samples contained <LOQ. Following 3x and 5x application rates, CGA-153433 was found at concentrations of 0.087-0.12 ppm only in straw samples from one trial at the 5X rate.

Table 12. Summary of Residue Data from Barley Field Trials with Cloquintocet-mexyl, as Safener in Herbicide NOA-407855 (MRID 46203204)

Commodity	Total Applic. Rate, (lb a.i./A)	PHI (days)	Residue Levels of CGA-153433 (ppm) [1]						
			n	Min.	Max.	HAFT[2]	Median (STMdR)	Mean (STMR)	Std. Dev.
CGA-153433 (metabolite of safener Cloquintocet-mexyl)									
Barley hay	0.016	30	24	<LOQ[3]	0.048	0.029	<LOQ	<LOQ	N/A[4]
Barley straw	0.016	60	24	<LOQ	0.050	0.036	<LOQ	<LOQ	N/A
Barley grain	0.016	60	24	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	N/A

[1] The method determines parent cloquintocet-mexyl and its metabolite CGA-153433 as CGA-153433.
[2] HAFT = Highest Average Field Trial.
[3] LOQ =0.01 ppm for grain and 0.02 ppm for hay and straw.
[4] N/A = not applicable.

MRID 46203205: Syngenta Crop Protection, Inc. has submitted field trial data for the safener cloquintocet-mexyl (CGA-185072) on barley. Residues of the safener metabolite CGA-153433 were measured on barley as part of a field trial for the new emulsifiable concentrate herbicide NOA-407855. One of three herbicide formulations was used on each plot (two variants of a 100 EC formulation and a 120 EC formulation). Three different adjuvants were also tested with each formulation. A total of 16 field trials were conducted in Canada in Zones 5, 5B, 7 (2), or 14 (12). Studies from MRID 46203205 with hay at PHIs of 26-35 days include Region 5B (1 study), Region 5 (1), Region 7 (1), and Region 14 (9). Studies from MRID 46203205 with straw and grain at PHIs of 57-66 days include Region 7 (1) and Region 14 (7).

Barley was grown under normal agricultural conditions on plots at each field trial location. The herbicide formulation was diluted with water for a targeted treatment rate of 17.5 g cloquintocet-mexyl/ha (0.016 lb/A) were applied. All treatments were made prior to emergence of the 4th tiller/3-6 leaf stage of barley, by one-time broadcast spray using 25-200 L/ha (2.7-21.4 gal/A). At normal commercial maturity, hay samples were cut approximately 26-48 days after treatment and straw and grain were harvested at 54-89 days after treatment. Hay was dried to 4.6-23.3% moisture content. In the decline study, hay samples were cut 7 and 14 days before and after normal harvest maturity and straw and grain samples were harvested 7 days prior and 7 and 14 days after normal harvest. Residues were not determined in processed grain fractions.

Analytical Method 199.02 was used to detect CGA-153433, the acidic metabolite of the safener additive cloquintocet-mexyl. During the acid refluxing of the sample, cloquintocet-mexyl is oxidized to CGA-153433. The LOQ, as presented in the method, is 0.01 ppm for each analyte for

grain and 0.02 ppm for hay and straw. The LOD was established to be ½LOQ. The quantitation of residues was based on LC-MS/MS peak area comparison with calibration standard solutions. Recovery samples were prepared at the LOQ, at 5X LOQ, and at 10X LOQ for each sample set. Average recoveries ranged from 83.6-106% (n=7-9) for CGA-153433.

Freezer storage time ranged from 38 to 139 days for all samples. Residues of the cloquintocet-mexyl have been shown to be stable in all matrices for the duration of storage that occurred during the conduct of this study. Minimal concentrations of the safener metabolite, CGA-153433, were found in hay. Only two hay samples (0.02 ppm) had residue levels equal to the LOQ and seven hay samples had levels above the LOD but below the LOQ. No safener residues were found in straw or grain. Decline of CGA-153433 could not be determined because all measured residues in the decline study were <LOQ.

Table 13. Summary of Residue Data from Barley Field Trials with Cloquintocet-mexyl, as Safener in Herbicide NOA-407855 (MRID 46203205)

Commodity	Total Applic. Rate, (lb ai/A)	PHI [1] (days)	Residue Levels of CGA-153433 (ppm) [2]						
			n	Min.	Max.	HAFT [3]	Median (STMdR)	Mean (STMR)	Std. Dev.
CGA-153433 (metabolite of safener cloquintocet-mexyl)									
Barley hay	0.016	26-35	24	<LOQ[4]	0.02	0.02	<LOQ	<LOQ	N/A [5]
Barley straw	0.016	57-66	20	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	N/A
Barley grain	0.016	57-66	20	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	N/A

[1] Studies from MRID 46203205 with hay at PHIs of 26-35 days include Region 5B (1 study), Region 5 (1), Region 7 (1), and Region 14 (9). Studies with straw and grain at PHIs of 57-66 days include Region 7 (1) and Region 14 (7).

[2] The method determines parent cloquintocet-mexyl and its metabolite CGA-153433 as CGA-153433.

[3] HAFT = Highest Average Field Trial

[4] LOQ = 0.01 ppm for hay and 0.02 ppm for grain and straw.

[5] N/A = not applicable

Conclusion. The submitted barley field trials are adequate in number and geographic representation. The data collection methods for barley determined combined residues of cloquintocet-mexyl and its metabolite CGA-153433 as CGA-153433, using an oxidation step to convert cloquintocet-mexyl to CGA-153433. Based on these residue data, residues are not expected to exceed 0.010 ppm in barley grain (LOQ) and 0.050 ppm in barley hay and straw. Because the EPA-validated enforcement methods determine parent and metabolite separately at LOQs of 0.05 ppm for each analyte on each commodity except for an LOQ of 0.02 ppm for parent in grain, the tolerances for the combined residues of cloquintocet-mexyl and its metabolite CGA-1532433 should be 0.10 ppm for barley, hay; 0.10 ppm for barley, grain; and 0.10 ppm for barley, straw.

860.1520 Processed Food and Feed

Wheat

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46203203.der.wpd

Deficiency #20 from D257181:

> Pending submission of storage stability data on CGA-153433 in processed commodities (see storage stability section of this review), HED concludes that no concentration of CGA-185072 or CGA-153422 occurred in processing.
>
> Note: The storage stability section of the D257181 review indicated that, pending submission of additional information requested for MRID 4439210, HED tentatively concluded that CGA-153433 was stable in wheat grain stored at -20°C for 727 days. The additional information requested for Study 300/91 (MRID 44399210) was given in Deficiency #17a of D257181: Raw data, including residues (ppm) found and representative chromatograms (for standards, controls, freshly fortified samples, and stored samples) should be submitted. Storage containers should be described. The method used to analzye the storage samples should be submitted or identified by number as a submitted method.
>
> Note: Deficiency #17c indicated that storage stability data were needed for CGA-153433 in wheat germ. Storage stability data were not requested in aspirated grain fractions since the use is an early season use and residues are not expected to occur in aspirated grain fractions. Storage stability data were not requested in bran, flour, middlings, and shorts since these matrices are similar to grain and can be covered by the storage stability data on grain.

Petitioner's Response: Syngenta Crop Protection, Inc. submitted a final report (MRID 46012904) of a processing study on wheat. In two trials conducted in ND (spring wheat) and OK (winter wheat), wheat grain (RAC) was harvested 59 or 61 days following a single broadcast foliar application of the 0.5 lb/gal EC formulation at 0.016 lb safener/A (7.1 g safener/A; 1x the field trial application rate) or 0.078 lb safener/A (35.5 g safener/A; 5x the field trial application rate); both application rates were used at both sites. Wheat grain was processed into germ, bran, middlings, shorts, and flour (including low grade and patent) using simulated commercial processing procedures. In addition, a sample of wheat aspirated grain fractions was generated for each trial site. An interim report of these wheat processing data has been reviewed previously (PP#7E04920; DP Barcode D257181, N. Dodd, 4/7/00); that submission included only the results from the processed commodities of the spring wheat samples.

Samples of wheat grain, processed commodities, and aspirated grain fractions were analyzed for residues of cloquintocet-mexyl using HPLC/UV Method REM 138.01, the current enforcement method for residues of cloquintocet-mexyl in wheat commodities. The reported LOQ was 0.02 ppm

for wheat grain and processed grain fractions. Samples were analyzed for residues of the metabolite CGA-153433 using HPLC/UV Method REM 138.10, the current enforcement method for residues of CGA-153433 in/on wheat commodities. The reported LOQ was 0.05 ppm for wheat grain and processed wheat commodities. These methods are adequate for data collection based on acceptable concurrent method recovery data.

The maximum storage intervals of processing study samples from harvest of wheat grain to analysis for cloquintocet-mexyl were 147 days (4.8 months) for wheat grain, 140 days (4.6 months) for aspirated grain fractions, and 153 days (5.0 months) for wheat processed commodities. The maximum storage intervals of processing study samples from harvest of grain to analysis for CGA-153433 were 159 days (5.2 months) for wheat grain, 161 days (5.3 months) for aspirated grain fractions and wheat processed fractions other than germ, and 158 days (5.2 months) for germ. The available storage stability data, submitted in conjunction with a previous cloquintocet-mexyl petition (PP#7E04920; DP Barcode D257181, 4/7/00, N. Dodd; MRID 44399208) demonstrate that residues of cloquintocet-mexyl are reasonably stable in wheat grain stored at -18 °C for up to ~6 months. Pending receipt of additional information regarding MRID 44399210, HED tentatively concluded that CGA-153433 is stable in wheat grain stored at -20 °C for 727 days (23.9 months). In addition, storage stability data submitted in conjunction with this submission (MRID 46012917) indicate that residues of CGA-153433 are reasonably stable in/on fortified samples of wheat germ for up to 165 days (5.4 months) of frozen storage.

Residues of cloquintocet-mexyl and its metabolite CGA-153433 were each below the limit of quantitation (LOQ; <0.02 ppm for cloquintocet-mexyl and <0.05 ppm for CGA-153433) in/on wheat grain treated with the 0.5 lb ai/gal EC formulation at 0.016 or 0.078 lb ai/A. Residues of cloquintocet-mexyl (parent) were below the LOQ (<0.02 ppm) in all samples of spring and winter wheat aspirated grain fractions and processed commodities (germ, bran, middlings, shorts, low grade flour, patent flour, and flour). Residues of CGA-153433 were also below the LOQ (<0.05 ppm) in all samples of spring and winter wheat aspirated grain fractions and processed commodities.

Although the processing factors were not calculated since all residues were below the LOQ's, this study (MRID 46012904) indicates that concentration on processing is not likely. According to Table 3 of OPPTS 860.1520, the theoretical concentration factors are 7.7x for wheat bran, 1.4x for wheat flour, and 8.3x for wheat shorts.

Syngenta submitted another processing study on wheat (MRID 46203203). Cloquintocet-mexyl (CGA-185072), as part of the NOA-407855 formulation 100EC, was applied to wheat in a single post foliar broadcast spray at 0.016 lb a.i./A (0.018 kg a.i./ha) for the 1X treatment and at 0.080 lb a.i./A (0.090 kg a.i./ha) for 5X treatment. Wheat was harvested 62 days after treatment. The wheat grain samples were processed into bran, flour, middlings, shorts, and germ. Residues in aspirated grain fractions were also determined.

Residues of metabolite CGA-153433 were quantified using Syngenta Analytical Method 117-01. The method converts all of the cloquintocet-mexyl to this acid metabolite. Quantification of residues was based on HPLC/MS/MS peak area comparisons. Satisfactory method performance in detecting CGA-153433 was demonstrated by concurrent recoveries. Freezer storage intervals ranged from 5.5-5.7 months after harvest for the processed commodities and for AGF. Previous studies (MRIDs 44399208 and 44399210) have shown that residues of the CGA-185072 are stable and tentatively (pending submission of additional information) residues of CGA-153433 are stable in/on wheat grain for the duration of frozen storage in the present study (up to 5.7 months).

CGA-153433 residues following both the 1X and 5X application rates were below the LOQ of 0.01 ppm in wheat grain and all processed commodities except AGF. In AGF, CGA-153433 residues were 0.012 ppm (at the1X application rate) and 0.071 ppm (at the 5X application rate); the processing factors are 1.2X and 7.1X, respectively, and averaged 4.2X. Based on the data in MRID 46203203, the residues in AGF could be calculated to be 0.042 ppm (0.01 ppm x 4.2x). According to Table 3 of OPPTS 860.1520, the theoretical concentration factors are 7.7x for wheat bran, 1.4x for wheat flour, and 8.3x for wheat shorts.

Conclusions. Deficiency #20 remains outstanding pending submission of additional information requested for Study 300/91 (MRID 44399210) regarding the storage stability data for grain. The additional information requested for Study 300/91 (MRID 44399210) are as follows: Raw data, including residues (ppm) found and representative chromatograms (for standards, controls, freshly fortified samples, and stored samples) should be submitted. Storage containers should be described. The method used to analyze the storage samples should be submitted or identified by number as a submitted method.

The submitted processing data for wheat are tentatively adequate to satisfy data requirements, pending submission of additional information requested for Study 300/91 (MRID 44399210) regarding the storage stability data for grain. The processing data indicate that residues of cloquintocet-mexyl and CGA-153433, determined as CGA-153433, do not concentrate in wheat processed commodities (bran, flour, middlings, shorts, and germ). Residues may concentrate in aspirated grain fractions (AGF) but residues in AGF are not likely to exceed the recommended tolerance of 0.10 ppm for grain. Therefore, tolerances are not needed for the wheat processed commodities (bran, flour, middlings, shorts, and germ) or for aspirated grain fractions.

Barley

46203204.der.wpd

A processing study was submitted for barley. Residues of CGA-153433, the metabolite of the safener cloquintocet-mexyl, were measured on barley processed fractions as part of a field trial for the new emulsifiable concentrate herbicide NOA-407855. Samples from a trial conducted in Jerome, ID were used to generate processed grain fractions (including flour, bran, and pearled barley) for determination of potential residue levels.

The herbicide formulation was diluted with water for a targeted treatment rate of 0.016 lb cloquintocet-mexyl/A (0.018 kg/ha; 1X) for grain. A treatment rate of 5X was also applied. All treatments were made by one-time post-foliar broadcast spray using 14-18 gallons/acre. Grain was harvested at 60 days after treatment.

Syngenta Analytical Method 117-01 was used for determination of residues of CGA-153433 in barley processed grain fractions with the LOQ at 0.01 ppm. The LOD was 0.005 ppm. The quantitation of residues was based on HPLC/MS/MS peak area comparison with calibration standard solutions. Concurrent recovery samples were prepared at the LOQ level up to 1 ppm for each processed fraction. Recoveries ranged from 84-105% with an average of 92% (n=8) for CGA-153433. Storage stability data for CGA-153433 on barley RAC were not included although limited data on wheat were available. Barley grain was stored 4.3-14.4 months; processed commodities were stored 4.3-14.9 months.

Residues of CGA-153433 were below the LOQ in all processed grain fractions even at the 5X herbicide application rate. A comparison of the residues in the RAC with those in each processed fraction showed no concentration of residues in processed fractions. The theoretical concentration factors for barley bran and pearled barley are 7.7 and 1.2, respectively.

Conclusions. Pending submission of additional information regarding storage stability of grain, HED tentatively concludes that the submitted barley processing study is adequate. Residues were <LOQ (<0.01 ppm) in barley grain treated at 5X and <LOQ (<0.01 ppm) in the processed fractions pearled barley, flour, and bran. Residues do not concentrate on processing and tolerances on the processed commodities pearled barley, flour, and bran are not needed.

860.1650 Submittal of Analytical Reference Standards

As of 10/13/04, analytical reference standards are available for cloquintocet-mexyl and its metabolite CGA-153433 at the EPA National Pesticide Standards Repository.

860.1850 Confined Accumulation in Rotational Crops

Deficiencies #'s 23 and 24 from D257181:

> 23. The submitted confined rotational crop data are adequate for a permanent tolerance provided that rotational crop restrictions are placed on the formulation label of at least 85 days (or 3 months) for lettuce and other leafy vegetables, 146 days (or 5 months) for small grains (except wheat), and one year (or 12 months) for all other crops.
>
> 24. If the petitioner wants shorter rotational crop restrictions, then a confined rotational crop study conducted at the soil aging intervals of 1, 4, and 12 months would be needed for three rotated crops (a small grain, a leafy vegetable, and a root crop) reflecting one application at the maximum label rate of 0.02 lb CGA-185072 safener/A.

Petitioner's Response to Deficiencies #'s 23 and 24:

In conjunction with a request for an SLN registration in North Dakota, an additional confined rotational crop study was submitted (MRID 45149902; DP Barcode D268038, 8/8/00, N. Dodd). In the study, [quinoline-3-^{14}C]cloquintocet-mexyl was applied to bare soil at 0.02 lb safener/A (1.3x the maximum seasonal rate), and representative rotational crops (mustard, turnip, and wheat) were planted at plantback intervals of 30 and 92 days, and wheat was planted at a plantback interval of 271 days. Total radioactive residues were found to be <0.002 ppm in/on all commodities at the 30-day plantback interval. Based on the results of the study, RAB3 concluded that a 30-day plantback interval is appropriate for cloquintocet-mexyl.

Conclusions:

Deficiencies #'s 23 and 24 are resolved. No additional data are needed for rotational crops. A 30-day plantback interval is appropriate for cloquintocet-mexyl for all crops not on the label. This 30-day (or higher) plantback restriction is included on the Discover®/Discover® NG Herbicide and Axial™ Herbicide labels.

The nature of the residue in rotational crops is adequately understood. The HED MARC previously determined for the purpose of the conditional registration that the residues of concern for the tolerance expression and risk assessment for rotational crops are cloquintocet-mexyl and its metabolite CGA-153433 (D263289, N. Dodd, 2/25/00). HED concludes that the residues of concern for the tolerance expression and risk assessment in rotational crops are cloquintocet-mexyl and its metabolite CGA-153433.

860.1900 Field Accumulation in Rotational Crops

Deficiency #25 from D257181:

> 25. No field accumulation in rotational crop study was submitted. Pending results from the confined rotational crop study which may be conducted if the petitioner wants shorter rotational crop restrictions, this study may be required.

Conclusion:

Deficiency #25 is resolved. Based on the available confined rotational crop data, the proposed rotational crop restrictions are appropriate. No field rotational crop data have been submitted but none are needed to support the requested uses on wheat and barley.

860.1550 Proposed Tolerances

The residues of concern for the tolerance expression and risk assessment are cloquintocet-mexyl and its metabolite CGA-153433, the residues that are currently regulated (40 CFR §180.560).

The available crop field trial data will support tolerances for residues of cloquintocet-mexyl and its metabolite CGA-153433 in/on wheat forage, hay, grain, and straw and on barley grain, hay, and straw. The established tolerances for wheat forage, hay, grain and straw should be retained and tolerances for barley grain, hay, and straw should be established based on the combined LOQs of the current enforcement methods. The available data indicate that the established tolerance levels for wheat commodities are appropriate.

There are currently no established Codex, Canadian, or Mexican MRLs for cloquintocet-mexyl. An International Residue Limit Status sheet is attached to this review. We note that registered food uses of clodinafop-propargyl exist in Canada (for wheat) and, therefore, cloquintocet-mexyl is used in Canada.

Table 14. Tolerance Summary for Cloquintocet-Mexyl.

Commodity	Established Tolerances (ppm)	Proposed Tolerances (ppm)	Recommended Tolerances (ppm)	Comments/ *Correct Commodity Definition*
Wheat, forage	0.1	0.2	0.10	
Wheat, grain	0.1	0.01	0.10	
Wheat, hay	0.1	0.5	0.10	
Wheat, straw	0.1	0.1	0.10	
Barley, grain	none	0.01	0.10	
Barley, hay	none	0.1	0.10	
Barley, straw	none	0.1	0.10	

Attachments:
Attachment 1: International Residue Limit Status sheet
Attachment 2: Appendix 1 - Chemical Name and Structure Table

Attachment 1

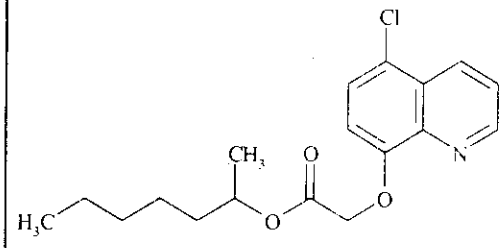
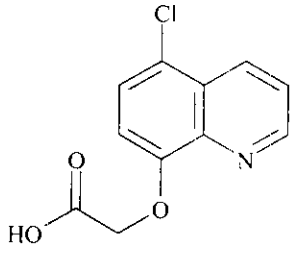
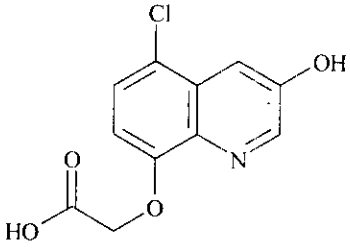
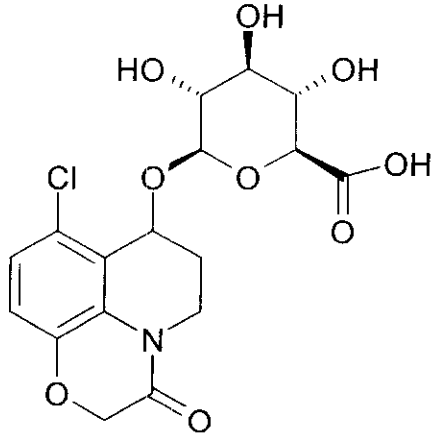
INTERNATIONAL RESIDUE LIMIT STATUS

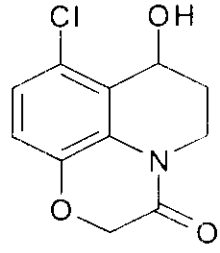
Chemical Name: acetic acid, [(5-chloro-8-quinolinyl)oxy]-, 1-methylhexyl ester)	Common Name: cloquintocet-mexyl	☒ Proposed tolerance ☐ Reevaluated tolerance ☐ Other	Date: 10/3/2005

Codex Status (Maximum Residue Limits)		**U. S. Tolerances**	
☐ No Codex proposal step 6 or above ☒ No Codex proposal step 6 or above for the crops requested		Petition Numbers: 7E04920, 4E06831 DP Barcode: D308470 Other Identifier:	
Residue definition (step 8/CXL):		Reviewer/Branch: Nancy Dodd, RAB3	
		Residue definition: cloquintocet-mexyl and its acid metabolite (5-chloro-8-quinolinoxyacetic acid)	
Crop (s)	**MRL (mg/kg)**	**Crop(s)**	**Tolerance (ppm)**
		wheat grain	0.10
		wheat forage	0.10
		wheat hay	0.10
		wheat straw	0.10
		barley grain	0.10
		barley hay	0.10
		wheat straw	0.10

Limits for Canada		**Limits for Mexico**	
☐ No Limits ☒ No Limits for the crops requested		☐ No Limits ☒ No Limits for the crops requested	
Residue definition:		Residue definition:	
Crop(s)	**MRL (mg/kg)**	**Crop(s)**	**MRL (mg/kg)**

Notes/Special Instructions: 11/03/05. SRFunk

Appendix I. Chemical Name and Structure of Cloquintocet-mexyl and its Transformation Products.		
Company Name	Chemical Name	Structure
Cloquintocet-mexyl	1-methylhexyl [(5-chloro-8-quinolinyl)oxy]acetate	
CGA-153433	[(5-chloroquinolin-8-yl)oxy]acetic acid	
OH-CGA-153433	[(5-chloro-3-hydroxyquinolin-8-yl)oxy]acetic acid	
M-1		

Appendix I. Chemical Name and Structure of Cloquintocet-mexyl and its Transformation Products.		
Company Name	Chemical Name	Structure
M-2		

Primary Evaluator: Nancy Dodd, Chemist
Registration Action Branch 3
Health Effects Division (7509C)
Date: 11/16/05

Approved by: William Wassell, Chemist
Registration Action Branch 3
Health Effects Division (7509C)
Date: 11/16/05

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 12/15/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

46012909 Muir, G.T.; Benner, J. P; and Kennedy, E. (2002) [Quinoline-3-^{14}C] - CGA-185072 Nature of the Residue in Spring Wheat. Lab Project Number: RJ3328B. Unpublished study submitted by Syngenta Crop Protection, Inc. 137 p.

EXECUTIVE SUMMARY:

Syngenta Crop Protection, Inc. has submitted a study investigating the metabolism of [quinoline-3-^{14}C]cloquintocet-mexyl (specific activity 55.2 µCi/mg) in spring wheat. The radiolabeled test substance was formulated as an emulsifiable concentrate formulation, mixed with Score® adjuvant and non-labeled clodinafop-propargyl (herbicide) and then diluted with water and applied to wheat plants, as a single foliar treatment, at nominal rates of 17.5 g safener/ha (0.016 lb safener/A) or 175.0 g safener/ha (0.16 lb safener/A). Applications were made to wheat plants at BBCH growth stages 22-30. Wheat forage was harvested 7 and 30 days after treatment, and wheat grain and straw were harvested at maturity, 61 days following treatment. The in-life and analytical phases of the study were conducted by Syngenta (Dewey, IL; Vero Beach, FL; Greensboro, NC; Jealott's Hill, UK; and Basel, Switzerland).

In wheat plants treated with [^{14}C]cloquinotcet-mexyl at 0.016 lb safener/A, total radioactive residues (TRR) were 0.438 ppm in forage harvested 7 days posttreatment, 0.019 ppm in forage harvested 30 days posttreatment, 0.004 ppm in mature straw, and 0.003 ppm in mature grain. In wheat plants treated with [^{14}C]cloquinotcet-mexyl at 0.16 lb safener/A, TRR were 3.004 ppm in forage harvested 7 days posttreatment, 0.204 ppm in forage harvested 30 days posttreatment, 0.029 ppm in mature straw, and 0.012 ppm in mature grain. Because of low radioactivity levels, samples of mature straw and grain from the low rate treatment were not subjected to extraction procedures. The majority of the TRR (~50-73%) was extracted from wheat forage using acetonitrile (ACN)/water; ACN/water released smaller amounts (29-35% TRR) from straw and grain. The petitioner subjected the ACN/water extract to cellulase hydrolysis and compared the chromatographic profile with that of the extract prior to hydrolysis. The nonextractable residues of 7-day PHI forage (both treatments) and 30-day PHI forage (high

rate treatment), which accounted for 29-36% TRR, were subjected to sequential microwave hydrolyses, using isopropanol/water, 0.5 N HCl, and 0.5 N NaOH to release additional residues, yielding 7-15% TRR in the isopropanol/water hydrolysate, 5-13% TRR in the 0.5 N HCl hydrolysate, and 6-14% TRR in the 0.5 N NaOH hydrolysate. The nonextractable residues of 30-day PHI forage (low rate treatment) and mature straw and grain (high rate treatment) accounted for 0.006 - 0.017 ppm (31.0% - 57.8% TRR) and were not subjected to additional characterization attempts. The accountabilities were 92-108%.

Residues were characterized/identified primarily by two-dimensional thin layer chromatographic (TLC) analyses, with confirmatory analysis by high performance liquid chromatography (HPLC). Liquid chromatography/mass spectrometry (LC/MS) and ^{1}H-nuclear magnetic resonance (^{1}H-NMR) analyses were used to identify the two major metabolites in 7-day PHI forage (high rate treatment). These methods successfully identified the predominant residues in wheat matrices. Adequate storage stability data were submitted, demonstrating the stability of the metabolic profiles in wheat forage for up to 26 months. Wheat grain and straw samples were analyzed within 3 months of sample collection; therefore, supporting storage stability data are not needed for these matrices.

Approximately 31-39% TRR were identified in forage, and 7% TRR were identified in mature straw. No metabolites were identified in mature wheat grain. Cloquintocet-mexyl was found in minor amounts in 7-day PHI forage only, at 3.4% TRR (0.015 ppm) and 2.2% TRR (0.066 ppm) in low and high rate studies, respectively. The metabolite OH-CGA-153433 was found to be the major metabolite, accounting for a total of 20.5-22.5% TRR (0.042-0.676 ppm) in forage (7-day PHI, both treatment rates, and 30-day PHI, high rate treatment) and 7.3% TRR (0.002 ppm) in mature straw (high rate treatment). Of these amounts, 11.9-12.9% TRR (0.026-0.388 ppm) was conjugated OH-CGA-153433 in forage (released after hydrolysis with cellulase). Metabolite CGA-153433 accounted for a total of 2.7-10.5% TRR (0.006-0.276 ppm) in forage; of these amounts, ~7% TRR was conjugated in 7-day PHI forage. Hydrolysis of bound residues yielded small amounts of CGA-153433 (1.8-3.7% TRR) and OH-CGA-153433 (1.6-4.4% TRR) in forage. A large portion of the remaining radioactivity consisted of unknowns; HPLC analyses of extracts and hydrolysates indicated that unknown peaks were each ≤4.6% TRR (≤0.020 ppm) in low rate treatment 7-day PHI forage, ≤5.0% TRR (≤0.150 ppm) in high rate treatment 7-day PHI forage, ≤3.7% TRR (≤0.008 ppm) in high rate treatment 30-day PHI forage, and ≤0.003 ppm in high rate treatment mature straw.

Based on the results of this study, the petitioner concluded that metabolism of cloquintocet-mexyl is rapid, since low levels were observed in 7-day PHI forage and it was not detected in 30-day PHI forage or mature grain and straw. Cloquintocet-mexyl undergoes de-esterification to form CGA-153433 which is hydroxylated to form OH-CGA-153433. Further metabolism results in the binding of these metabolites to crop matrix and the generation of multiple polar components.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the wheat metabolism data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode 308470.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would impact the validity of the study.

A. BACKGROUND INFORMATION

The safener cloquintocet-mexyl is included in systemic herbicide formulations to prevent damage to wheat plants from the phytotoxic effects of the herbicide. Discover® Herbicide/Discover NG® Herbicide, containing cloquintocet-mexyl and the active ingredient clodinafop-propargyl (CGA-184927), were conditionally registered for use on wheat in June 2000 (PP#7E04920; DP Barcode 257181, 4/7/00, N. Dodd). The petitioner has now submitted data to satisfy the conditions of full registration.

Syngenta has also petitioned for amended tolerances to support additional use of cloquintocet-mexyl as a safener in a pesticide formulation (Axial™ Herbicide) containing the new active ingredient pinoxaden for use on both wheat and barley. In connection with the pinoxaden petition, Syngenta proposed to revise the established tolerances for cloquintocet-mexyl residues in/on wheat commodities and proposed tolerances on barley commodities (PP#4E06831; 69 FR 31118, 6/2/04; and 69 FR 67731, 11/19/04).

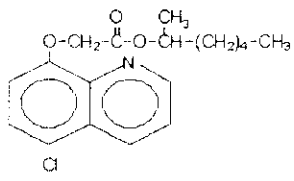
TABLE A.1. Test Compound Nomenclature	
Compound	Chemical Structure 
Common name	Cloquintocet-mexyl
Company experimental name	CGA-185072
IUPAC name	(5-chloroquinolin-8-yloxy)acetic acid 1-methylhexyl ester
CAS name	acetic acid, [(5-chloro-8-quinolinyl)oxy]-, 1-methylhexyl ester
CAS #	99607-70-2
End-use products/EPs	Discover® Herbicide (EPA Reg. No. 100-907) Discover® NG Herbicide (EPA Reg. No. 100-1173) Axial™ Herbicide (EPA Reg. No. 100-XXX)

TABLE A.2. Physicochemical Properties of the Technical Grade of Cloquintocet-mexyl		
Parameter	Value	Reference
Melting range	61.4 to 69°C	MRID 44387401
pH	5.4 at 25°C (1% w/v aqueous disp.)	MRID 44387401
Density	1.05 g/cm^3 at 22°C	MRID 44387401
Water solubility (25°C)	0.59 mg/L at pH 7.0 (PAI)*	MRID 44387401
Solvent solubility (g/L) (25°C)	ethanol - 190 acetone - 340 toluene - 360 n -hexane - 0.140 n-octanol - 11	MRID 44387401
Vapor pressure at 25°C	3.98 x10^{-8} mm Hg (PAI)*	MRID 44387401
Dissociation constant (pK_a)	3.55 (PAI)*	MRID 44387401
Octanol/water partition coefficient log P_{ow}	5.03 (at 25°C) (PAI)*	MRID 44387401
UV/visible absorption spectrum	Two absorbance maxima occur at 243.8-255.8 nm and 317.6-364.0 nm. No absorbance maxima occur between 370 nm and 750 nm.	MRID 44387401

*PAI = pure active ingredient

B. EXPERIMENTAL DESIGN

B.1. Test Site and Crop Information

TABLE B.1.1. Test Site Information				
Testing Environment	Soil characteristics			
	Type	% OM	pH	CEC
Outdoor test plots (5 x 12 ft) at Syngenta Midwest Research Station (Dewey, IL)	silty clay loam	3.6	6.4	18.8

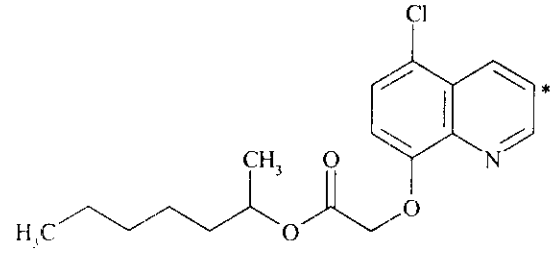
The petitioner stated that temperature and rainfall during the study period were typical for the testing location and season. Minor crop injury was noted 7 days following test substance application; some of this injury was due to high winds. No other evidence of phytotoxicity was observed during the study.

The control plot used for this study was the control plot used for the clodinafop-propargyl wheat metabolism study (see DER for MRID 46012908; 46012908.der).

TABLE B.1.2. Crop Information.					
Crop/crop group	Variety	Growth stage at application	Growth stage at harvest	Harvested RAC	Harvesting procedure
Spring wheat	Cultivar '2375'	Plant height of 4-6 inches (Feekes growth stage 4-6; BBCH stage 22-30)	Immature - 7 days post-treatment	Forage	Forage samples were collected by hand using scissors to cut entire above-ground portion of plant. Mature wheat was harvested using a knife and threshed to separate into straw plus husks[1] and grain.
			Immature - 30 days post-treatment	Forage	
			Mature	Straw (including husks)	
				Grain	

[1] The petitioner refers to straw plus husks as fodder.

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics	
Chemical structure	
Radiolabel position	[quinoline-3-^{14}C]cloquintocet-mexyl
Lot No.	GAN-XLV-42-1
Purity	99.0% (radiochemical; determined by TLC)
Specific activity	55.2 µCi/mg (2.042 MBq/mg)

B.3. Study Use Pattern

TABLE B.3.1. Use Pattern Information	
Chemical name	[quinoline-3-^{14}C]cloquintocet-mexyl
Application method	The test substance was mixed with 240 EC formulation blank, Score® adjuvant, and a non-labeled clodinafop-propargyl and then diluted with water and applied to wheat plants as a foliar treatment using an R&D CO_2-powered sprayer.
Application rate	0.016 lb safener/A (17.5 g safener/ha) or 0.16 lb safener/A (175.0 g safener/ha) (nominal rates)
Number of applications	One
Timing of applications	Postemergence, 33 days after planting
PHI	61 days for mature wheat; 7 and 30 days for forage from immature plants

B.4. Identification/Characterization of Residues

B.4.1. Sample Handling and Preparation

Samples of wheat matrices were frozen after collection and then shipped frozen to Syngenta Vero Beach Research Center (Vero Beach, FL) for homogenization and radioanalysis; homogenization was conducted in the presence of dry ice. Samples were then shipped frozen to Syngenta Crop Protection (Greensboro, NC), where they were stored frozen (~-20 °C) until analysis. Extracts were stored frozen or refrigerated (~4 °C) until analysis.

Crop samples and extracts were later shipped frozen to Syngenta (Jealott's Hill, UK) for further analyses. At Jealott's Hill, samples were stored frozen at -32 to -10 °C prior to analysis.

Samples of 7- and 30-day PHI wheat forage from the low rate treatment and samples of 7- and 30-day PHI wheat forage and mature wheat grain and straw from the high rate treatment were subjected to extraction procedures to characterize residues. Because of low residue levels, samples of wheat straw and grain from the low rate treatment were not subjected to extraction procedures. In general, samples were extracted by homogenization in acetonitrile:water (8:2, v:v; 3x). Extracts were isolated by filtration and combined. Extracts were cleaned up by C-18 solid-phase extraction (SPE), allowing the sample to pass through the column, washing the column with acetonitrile/water (80:20, v:v), and then rinsing the column with additional acetonitrile (ACN). The C-18 SPE eluates were reserved for TLC and HPLC analysis. Extracts were then concentrated and subjected to enzyme hydrolysis using cellulase (in 0.1 M sodium acetate buffer, pH 4.6, at 50 °C for approximately 48 hours; additional cellulase was added after 24 hours of incubation). The cellulase hydrolysate was reserved for TLC and HPLC analysis.

The nonextractable residues of 7-day PHI forage (both treatment rates) and 30-day PHI forage (high rate treatment) were subjected to microwave assisted extraction using, in sequence, isopropanol:water (8:2, v:v), 0.5 N HCl, 6 N HCl (7-day PHI forage from low application rate only), and 0.5 N NaOH. In each case, samples were heated at 100 °C for 16 minutes, then at 120 °C for 12 minutes, and then at 150 °C for 22 minutes. The extract was isolated by filtration. For 7-day PHI low rate forage, the isopropanol/water hydrolysate was cleaned up by C-18 SPE. The solvents used for SPE were not specified, but cleanup yielded an aqueous fraction and the combined C18 washes; the wash fraction was reserved for HPLC analysis. The 0.5 N HCl hydrolysate was partitioned with methyl t-butyl ether (MTBE) and ethyl acetate, and the aqueous fraction was applied to a C-18 SPE column, using methanol and 1% HCl in methanol to elute the column; the methanol eluates were combined with the organic phase from partitioning and reserved for TLC and HPLC analysis. The 0.5 N NaOH hydrolysate was partitioned with MTBE and ethyl acetate; the resulting fractions were combined.

For 7-day PHI high rate forage, the isopropanol/water hydrolysate was cleaned up by C-18 SPE. The solvents used for SPE were not specified, but cleanup yielded an isopropanol/water fraction and a chloroform fraction. The isopropanol/water eluate was further cleaned up by C-18 SPE, yielding an aqueous fraction, a methanol fraction, a 1% HCl in methanol fraction, and a

chloroform fraction; the methanol fractions were combined and reserved for TLC and HPLC analysis, and the chloroform fraction was combined with the chloroform fraction from the first C-18 SPE cleanup. The 0.5 N HCl hydrolysate was cleaned up by C-18 SPE, yielding an aqueous fraction, a methanol fraction, and a 1% HCl in methanol fraction; the methanol fractions were combined and reserved for TLC and HPLC analysis. The 0.5 N NaOH hydrolysate was reserved for TLC and HPLC analysis.

For 30-day high rate forage, the isopropanol/water and 0.5N HCl hydrolysates were separately mixed with ammonium acetate buffer and concentrated for TLC and/or HPLC analysis. The 0.5 N NaOH hydrolysate was split into two subsamples: subsample 1 was acidified to pH 2, mixed with water, and cleaned up by C-8 SPE, yielding an aqueous fraction and a methanol fraction; and subsample 2 was neutralized with HCl, concentrated, mixed with ammonium acetate buffer, acidified to pH 2, mixed with water, and cleaned up by C-8 SPE, yielding an aqueous fraction and a methanol fraction. The methanol fractions from the two subsamples were combined and reserved for HPLC analysis.

B.4.2. Analytical Methodology

Total radioactive residues (TRR) in wheat matrices were determined by combustion/liquid scintillation counting (LSC). Extracts and hydrolysates were radioassayed by LSC, and nonextractable residues were radioassayed by combustion/LSC. The limit of detection was 0.001 ppm.

Sample extracts/hydrolysates were analyzed by two-dimensional TLC and/or HPLC. TLC analyses were conducted on silica gel 60 F-254 plates (normal phase) using ethyl acetate:methanol:acetic acid:water (62:24:2:11, v:v:v:v) in one dimension and chloroform:methanol:formic acid:water (82:17:5:2.5, v:v:v:v) in the second dimension. Radioactivity was detected using an imaging system. Metabolites were identified by cochromatography with reference standards; the chemical names and structures of the reference standards used are presented in Appendix I.

HPLC analyses were conducted to confirm metabolite identifications and to profile certain hydrolysate extracts, using a system equipped with a UV detector (254 nm), a radiodetector, a fraction collector, and a C-8 column, and using a gradient mobile phase of acetonitrile and pH 6 ammonium acetate in water. Metabolites were identified by comparison of retention times with those of the reference standards. Radioactive areas were quantified using fraction collection/LSC. Preparative HPLC was also used for isolation of CGA-153433 and OH-CGA-153433 for identification, using a C-18 column and an isocratic mobile phase of acetonitrile and water, each containing 0.5% trifluoroacetic acid.

C. RESULTS AND DISCUSSION

The storage intervals and conditions for the wheat metabolism study are presented in Table C.1. The petitioner stated that all samples were combusted within 51 days of collection,

extracted within 78 days of collection, and initial chromatographic profiles were obtained within 81 days of collection. Only forage samples were subjected to extraction/analyses beyond the initial chromatographic profiles. No final analysis dates were reported for forage samples. Based on the date provided for completion of storage stability analyses, which were reportedly conducted at the end of the study, forage samples were stored for up to 26 months prior to completion of analysis. The TLC analyses of forage extracts which were conducted at the end of the study indicate that the metabolite profile did not change significantly in forage extracts over the course of the study. These data are adequate to support the wheat metabolism study. Because wheat grain and straw samples were analyzed within 3 months of sample collection, supporting storage stability data are not needed for these matrices.

Total radioactive residues (TRR) in spring wheat forage, grain, and straw are reported in Table C.2.1. In wheat plants treated with [quinoline-3-^{14}C]cloquinotcet-mexyl at 0.016 lb safener/A, TRR were 0.438 ppm in forage harvested 7 days posttreatment, 0.019 ppm in forage harvested 30 days posttreatment, 0.004 ppm in mature straw, and 0.003 ppm in mature grain. In wheat plants treated with [quinoline-3-^{14}C]cloquinotcet-mexyl at 0.16 lb safener/A, TRR were 3.004 ppm in forage harvested 7 days posttreatment, 0.204 ppm in forage harvested 30 days posttreatment, 0.029 ppm in mature straw, and 0.012 ppm in mature grain.

The distribution of the radioactivity in wheat matrices is presented in Tables C.2.2.1 (low rate treatment) and C.2.2.2 (high rate treatment). Because of low radioactivity levels, samples of mature straw and grain from the low rate treatment were not subjected to extraction procedures. The majority of the TRR (~50-73%) was extracted from wheat forage using ACN/water; ACN/water released smaller amounts (29-35% TRR) from straw and grain. The petitioner subjected the ACN/water extract to cellulase hydrolysis and compared the chromatographic profile with that of the extract prior to hydrolysis. The nonextractable residues of 7-day PHI forage (both treatments) and 30-day PHI forage (high rate treatment), which accounted for 29-36% TRR, were subjected to sequential microwave hydrolyses, using isopropanol/water, 0.5 N HCl, and 0.5 N NaOH to release additional residues, yielding 7-15% TRR in the isopropanol/water hydrolysate, 5-13% TRR in the 0.5 N HCl hydrolysate, and 6-14% TRR in the 0.5 N NaOH hydrolysate. The nonextractable residues of 30-day PHI forage (low rate treatment), and mature straw and grain (high rate treatment) accounted for 0.006-0.017 ppm and were not subjected to additional characterization attempts. The accountabilities were 92-108%. Residues were characterized/identified primarily by two-dimensional TLC analyses, with confirmatory analysis by HPLC. These methods successfully identified the predominant residues in wheat matrices.

The characterization and identification of residues in the wheat matrices in which metabolites were identified, 7-day PHI forage (both treatment rates), 30-day PHI forage (high rate treatment), and mature straw (high rate treatment), are summarized in Table C.2.3. Approximately 31-39% TRR were identified in forage, and 7% TRR were identified in mature straw. Cloquintocet-mexyl was found in minor amounts in 7-day PHI forage only, at 3.4% TRR (0.015 ppm) and 2.2% TRR (0.066 ppm) in low and high rate studies, respectively. The metabolite OH-CGA-153433 was found to be the major metabolite, accounting for a total of

20.5-22.5% TRR (0.042-0.676 ppm) in forage (7-day PHI, both treatment rates, and 30-day PHI, high rate treatment) and 7.3% TRR (0.002 ppm) in mature straw (high rate treatment). Of these amounts, 11.9-12.9% TRR (0.026-0.388 ppm) was conjugated OH-CGA-153433 in forage (released after hydrolysis with cellulase). Metabolite CGA-153433 accounted for a total of 2.7-10.5% TRR (0.006-0.276 ppm) in forage; of these amounts, ~7% TRR was conjugated in 7-day PHI forage. Hydrolysis of bound residues yielded small amounts of CGA-153433 (1.8-3.7% TRR) and OH-CGA-153433 (1.6-4.4% TRR) in forage. A large portion of the remaining radioactivity consisted of unknowns; HPLC analyses of extracts and hydrolysates indicated that unknown peaks were each ≤4.6% TRR (≤0.020 ppm) in low rate treatment 7-day PHI forage, ≤5.0% TRR (≤0.150 ppm) in high rate treatment 7-day PHI forage, ≤3.7% TRR (≤0.008 ppm) in high rate treatment 30-day PHI forage, and ≤0.003 ppm in high rate treatment mature straw.

One major unknown metabolite was observed in the crop commodities, and a subsample of 7-day PHI forage from the high rate treatment was used to isolate the metabolite; metabolite CGA-153433 was also isolated using this procedure. The subsample was sequentially extracted with hexane, dichloromethane, ACN, ACN:water (1:1, v:v), and water. The ACN, ACN/water, and water fractions, which contained the most radioactivity, were combined, cleaned up by C-18 SPE, and hydrolyzed with cellulase (as described above). The hydrolysate was adjusted to pH 2 using HCl, and partitioned with ethyl acetate. TLC analysis of the ethyl acetate fraction indicated that CGA-153433 and the unknown were contained in that fraction. The fraction was evaporated to dryness, redissolved in ACN:water (2:8, v:v) and then purified using preparative HPLC. The isolated fractions were sent to Syngenta Crop Protection in Basel, Switzerland where the metabolites were identified using ^{1}H-NMR spectroscopy and mass spectrometry. The isolated metabolites were analyzed using an LC-NMR system, consisting of a C-18 column and a gradient mobile phase of acetonitrile and deuterated water and a 600 MHZ NMR spectrometer. LC/MS analyses of the isolated metabolites were conducted using electrospray ionization in the positive ion mode. Based on the analyses, the metabolites were identified as CGA-153433 and OH-CGA-153433.

The petitioner noted that cellulase treatment of ACN/water extracts appeared to result in the conversion of cloquintocet-mexyl to CGA-153433. To test this possibility, cloquintocet-mexyl reference standard was combined with the buffer solution used for cellulase hydrolysis and then incubated at 50 °C for 89 hours. TLC analyses of the resulting sample showed partial breakdown of cloquintocet-mexyl to CGA-153433; a quantitative value for the amount of breakdown could not be determined because of the method used for analysis.

We note that the submission included limited raw data. Quantitative data tables for TLC and HPLC analyses were provided for selected extracts only, and only representative chromatograms were provided. In many cases, quantitative data for the analyses of hydrolysates were only provided in the text. For future submissions, the petitioner should note that quantitative data tables for all analyses should be included, preferably along with the chromatograms.

C.1. Storage Stability

Samples of wheat matrices were frozen after collection and then shipped frozen to Syngenta Vero Beach Research Center (Vero Beach, FL) for homogenization and radioanalysis. Samples were then shipped frozen to Syngenta Crop Protection (Greensboro, NC), where they were stored frozen (~-20 °C) until analysis. Extracts were stored frozen or refrigerated (~4 °C) until analysis. Crop samples and extracts were later shipped frozen to Syngenta (Jealott's Hill, UK) for further analyses. At Jealott's Hill, samples were stored frozen at -32 to -10 °C prior to analysis. It could not be determined from the submission which extractions/analyses were conducted at Greensboro and which were conducted at Jealott's Hill.

All samples were combusted within 51 days of collection, extracted within 78 days of collection, and initial chromatographic profiles were obtained within 81 days of collection. Storage stability data are not required for metabolism samples analyzed within six months of harvest.

Although crops (wheat forage, grain, and straw) were extracted and profiled chromatographically within 6 months of harvest, additional analyses were conducted on 1x rate 7-day forage, 10x rate 7-day forage, and 10x rate 30-day forage after this 6-month period. To support storage stability of these samples, these samples were reanalyzed chromatographically, with analyses completed 26 months after harvest. The petitioner reported that qualitative comparison of the profiles indicated that no significant changes occurred in the profiles during the storage period. Only one pair of chromatograms (10x rate 7-day forage extracts analyzed in July 2000 and July 2002; Figure 23 of MRID 46012909) were submitted; these chromatograms indicated that some loss of residues may have occurred but no additional metabolites were present.

TABLE C.1. Summary of Storage Conditions.

Matrix	Storage Temp. (°C)	Actual Storage Duration	Interval of Demonstrated Storage Stability
Wheat treated at 0.016 lb safener/A			
Forage, 7-day PHI	~-20	up to 26 months	26 months
Forage, 30-day PHI		up to 26 months	
Mature straw		36 days	Not applicable; samples were analyzed within 6 months of collection.
Mature grain		36 days	
Wheat treated at 0.16 lb safener/A			
Forage, 7-day PHI	~-20	up to 26 months	26 months
Forage, 30-day PHI		up to 26 months	
Mature straw		81 days	Not applicable; samples were analyzed within 6 months of collection.
Mature grain		81 days	

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1. Total Radioactive Residues (TRR) in Wheat Matrices.

Matrix	Application rate (lb ai/A)	PHI (days)	ppm, [^{14}C]cloquintocet-mexyl equivalents
Forage	0.016	7	0.438
		30	0.019
	0.16	7	3.004
		30	0.204
Straw	0.016	61	0.004
	0.16	61	0.029
Grain	0.016	61	0.003
	0.16	61	0.012

TABLE C.2.2.1. Distribution of the Parent and the Metabolites in Wheat Matrices Following Application of [Quinoline-3-^{14}C]Cloquintocet-mexyl at 0.016 lb Safener/A.[1]

Metabolite Fraction	7-day PHI Forage		30-day PHI Forage	
	TRR = 0.438 ppm		TRR = 0.019 ppm	
	% TRR	ppm	% TRR	ppm
ACN/water	63.9	0.280	55.8	0.011
C-18 SPE ACN/water	59.0	0.258	55.0 [2]	0.010
Cloquintocet-mexyl	3.4	0.015		
CGA-153433	3.5	0.015		
OH-CGA-153433	8.9	0.039		
Unknown	3.0	0.013		
Unresolved	18.6 [3]	0.082		
Origin	21.5 [3]	0.094		
ACN/water cellulase hydrolysate	53.3	0.234	47.8 [4]	0.009
CGA-153433	10.5 [5]	0.046		
OH-CGA-153433	20.8	0.091		
Unknowns	22.0 [6]	0.096		
Solids	32.6	0.143	31.0	0.006
*i*PrOH:water microwave extract	11.1	0.049		
C-18 SPE aqueous	1.6	0.007		
C-18 SPE washes	7.1	0.031		
CGA-153433	0.9	0.004		
OH-CGA-153433	0.7	0.003		
Unknowns	5.5 [7]	0.024		
Solids	NR	NR		
0.5 N HCl hydrolysate	6.0	0.026		

TABLE C.2.2.1. Distribution of the Parent and the Metabolites in Wheat Matrices Following Application of [Quinoline-3-^{14}C]Cloquintocet-mexyl at 0.016 lb Safener/A.[1]

Metabolite Fraction	7-day PHI Forage		30-day PHI Forage	
	TRR = 0.438 ppm		TRR = 0.019 ppm	
	% TRR	ppm	% TRR	ppm
Combined organic fractions	4.6	0.020		
CGA-153433	0.9	0.004		
OH-CGA-153433	0.9	0.004		
Unknowns	2.8 [8]	0.012		
Aqueous fraction	1.4	0.006		
Solids	NR	NR		
6.0 N HCl hydrolysate	0.7	0.003		
Solids	NR	NR		
0.5 N NaOH	6.2	0.027		
Combined aqueous and organic fractions	3.7	0.016		
Solids	6.2	0.027		

[1] Extracts were analyzed by two-dimensional (2D) TLC; identification of metabolites in major extracts was confirmed by HPLC. For the hydrolysates of nonextractable residues, metabolites were identified by comparison of HPLC retention times with those of reference standards. Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question. NR = Not reported.

[2] HPLC analysis of this fraction indicated the presence of CGA-153433 and OH-CGA-153433 at low levels (~0.001 ppm) plus six polar components, each ≤0.002 ppm.

[3] HPLC analysis indicated the presence of at least 12 unknowns, each ≤9.8% TRR (≤0.043 ppm).

[4] HPLC analysis of this fraction indicated the presence of CGA-153433 (0.001 ppm) and OH-CGA-153433 (0.003 ppm) plus seven polar components, each ≤0.001 ppm.

[5] The petitioner noted that because cloquintocet-mexyl appeared to convert to CGA-153433 under the conditions used for cellulase hydrolysis, a portion of the reported CGA-153433 levels in the hydrolysate were due to cloquintocet-mexyl.

[6] HPLC analysis indicated the presence of at least 10 unknowns, each ≤4.6% TRR (≤0.020 ppm).

[7] HPLC analysis indicated the presence of nine unknowns, each ≤1.6% TRR (≤0.007 ppm). The %TRR and ppm value for this fraction was calculated by difference, as the petitioner did not report the total amount of the unknowns.

[8] HPLC analysis indicated the presence of six unknowns, each ≤0.6% TRR (≤0.003 ppm). The %TRR and ppm value for this fraction was calculated by difference, as the petitioner did not report the total amount of the unknowns.

TABLE C.2.2.2. Distribution of the Parent and the Metabolites in Wheat Matrices Following Application of [Quinoline-3-^{14}C]Cloquintocet-mexyl at 0.16 lb Safener/A.[1]

Metabolite Fraction	7-day PHI Forage		30-day PHI Forage		Mature Straw		Mature Grain	
	TRR = 3.004 ppm		TRR = 0.204 ppm		TRR = 0.029 ppm		TRR = 0.012 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
ACN/water	72.9	2.190	50.3	0.103	34.6	0.010	29.0	0.003
C-18 SPE ACN/water	67.3	2.022	46.4	0.095	36.2	0.010	27.4 [2]	0.003
Cloquintocet-mexyl	2.2	0.066	--	--	--	--		
CGA-153433	2.0	0.060	4.7	0.010	--	--		
OH-CGA-153433	9.6	0.288	7.8	0.016	7.3	0.002		
Unknowns	2.9	0.087	33.9 [3,4]	0.069	--	--		
Unassigned	23.1 [5]	0.694	--	--	--	--		

TABLE C.2.2.2. Distribution of the Parent and the Metabolites in Wheat Matrices Following Application of [Quinoline-3-^{14}C]Cloquintocet-mexyl at 0.16 lb Safener/A.[1]

Metabolite Fraction	7-day PHI Forage		30-day PHI Forage		Mature Straw		Mature Grain	
	TRR = 3.004 ppm		TRR = 0.204 ppm		TRR = 0.029 ppm		TRR = 0.012 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Origin	27.7 [5]	0.832	--	--	28.9 [6]	0.008		
ACN/water cellulase hydrolysate	61.4	1.845	41.5	0.085	33.8 [7]	0.010	23.8 [2]	0.003
CGA-153433	11.4 [8]	0.342	2.7	0.006				
OH-CGA-153433	22.5	0.676	20.5	0.042				
Unknowns	--	--	1.0	0.002				
Unassigned	8.5 [9]	0.255	--	--				
Origin	17.3 [9]	0.520	17.3 [10]	0.035				
Solids	28.7	0.861	35.5	0.072	57.8	0.017	72.0	0.009
*i*PrOH:water microwave extract	14.5	0.438	6.6	0.013				
Concentrate for analysis			5.0	0.010				
CGA-153433			1.0	0.002				
OH-CGA-153433			0.9	0.002				
Unknowns			3.1 [4,11]	0.006				
C-18 SPE *i*PrOH:water	11.6	0.348						
C-18 SPE aqueous	0.2	0.006						
C-18 SPE methanol	8.9	0.267						
C-18 SPE 1% HCl methanol	1.6	0.048						
Combined methanol	10.2	0.306						
CGA-153433	1.5	0.045						
OH-CGA-153433	1.6	0.048						
Unknowns	7.1 [4,12]	0.213						
C-18 SPE chloroform	0.8	0.024						
C-18 SPE chloroform	2.0	0.060						
Combined chloroform	3.1	0.093						
Solids	NR	NR	NR	NR				
0.5 N HCl hydrolysate	5.0	0.150	12.8	0.026				
Concentrate for analysis			8.6	0.018				
CGA-153433			1.7	0.003				
OH-CGA-153433			2.2	0.004				
Unknowns			4.7 [4,13]	0.011				
C-18 SPE aqueous	1.2	0.038						
C-18 SPE methanol	2.5	0.075						

TABLE C.2.2.2. Distribution of the Parent and the Metabolites in Wheat Matrices Following Application of [Quinoline-3-^{14}C]Cloquintocet-mexyl at 0.16 lb Safener/A.[1]

Metabolite Fraction	7-day PHI Forage		30-day PHI Forage		Mature Straw		Mature Grain	
	TRR = 3.004 ppm		TRR = 0.204 ppm		TRR = 0.029 ppm		TRR = 0.012 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
C-18 SPE 1% HCl methanol	1.4	0.042						
Combined methanol	3.7 [14]	0.111						
Solids	NR	NR	NR	NR				
0.5 N NaOH hydrolysate	14.1	0.424	14.4	0.029				
C-8 SPE aqueous, subsample 1			0.4	0.001				
C-8 SPE aqueous, subsample 2			0.6	0.001				
Combined C-8 SPE methanol, subsamples 1 and 2			11.1	0.023				
CGA-153433	1.2	0.036	1.0	0.002				
OH-CGA-153433	1.0	0.030	1.3	0.003				
Unknowns	11.9 [4,15]	0.358	8.8 [4,16]	0.018				
Solids (+ filter paper)	1.4	0.042	8.2	0.017				

[1] Extracts were analyzed by two-dimensional (2D) TLC; identification of metabolites in major extracts was confirmed by HPLC. For the hydrolysates of nonextractable residues, metabolites were identified by comparison of HPLC retention times with those of reference standards. Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question. NR = Not reported.

[2] 2D TLC analysis of the unhydrolyzed extract indicated that the radioactivity was associated with highly polar material; HPLC analysis indicated the presence of five unknowns. 2D TLC and HPLC analyses of the hydrolyzed extracts indicated no changes in the profiles upon hydrolysis.

[3] HPLC analysis indicated the presence of nine polar unknowns, each ≤9.7% TRR (≤0.020 ppm).

[4] The %TRR and ppm value for this fraction was calculated by difference, as the petitioner did not report the total amount of the unknowns.

[5] HPLC analysis indicated that unassigned/origin radioactivity in 2D TLC profile consisted of at least 14 unknowns, each ≤9.7% TRR (≤0.291 ppm).

[6] Radioactivity remained at the origin when analyzed by 2D TLC; HPLC analysis indicated the presence of three unknowns, each ≤0.003 ppm.

[7] Radioactivity remained at the origin when analyzed by 2D TLC; HPLC analysis indicated the presence of trace amounts of CGA-153433 (<0.001 ppm) and OH-CGA-153433 (0.001 ppm) plus five unknown peaks (each ≤0.003 ppm). The petitioner stated that the HPLC profile was qualitatively similar to the profile of the unhydrolyzed extract.

[8] The petitioner noted that because cloquintocet-mexyl appeared to convert to CGA-153433 under the conditions used for cellulase hydrolysis, a portion of the reported CGA-153433 levels in the hydrolysate were due to cloquinocet-mexyl.

[9] HPLC analysis indicated that unassigned/origin radioactivity in 2D TLC profile consisted of at least 15 unknowns, each ≤5.0% TRR (≤0.150 ppm).

[10] HPLC analysis indicated at least 11 peaks, each ≤3.7% TRR (≤0.008 ppm).

[11] HPLC analysis indicated the presence of five unknowns, each ≤1.3% TRR (≤0.003 ppm).

[12] HPLC analysis indicated the presence of 11 unknowns, each ≤2.0% TRR (≤0.060 ppm).

[13] HPLC analysis indicated the presence of seven unknowns, each ≤0.7% TRR (≤0.001 ppm).

[14] HPLC analysis indicated 10 areas of radioactivity, each ≤0.7% TRR (≤0.021 ppm).

[15] HPLC analysis indicated the presence of nine unknowns, each ≤3.6% TRR (≤0.108 ppm).

[16] HPLC analysis indicated the presence of four unknowns, each ≤2.2% TRR (≤0.004 ppm).

TABLE C.2.3. Summary of Characterization and Identification of Radioactive Residues in Plant Matrices Following Application of [Quinoline-3-^{14}C]Cloquintocet-mexyl at 0.016 or 0.16 lb Safener/A.

Compound	0.016 lb safener/A		0.16 lb safener/A					
	7-day PHI Forage		7-day PHI Forage		30-day PHI Forage		Mature Straw	
	TRR = 0.438 ppm		TRR = 3.004 ppm		TRR = 0.204 ppm		TRR = 0.029 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Extractable residues:								
Cloquintocet-mexyl	3.4	0.015	2.2	0.066	--	--	--	--
CGA-153433, total	10.5	0.046	9.2	0.276	2.7	0.006	--	--
CGA-153433, conjugated	7.0	0.031	7.2	0.216	--	--	--	--
OH-CGA-153433, total	20.8	0.091	22.5	0.676	20.5	0.042	7.3	0.002
OH-CGA-153433, conjugated	11.9	0.052	12.9	0.388	12.7	0.026	--	--
Unknowns/unassigned/origin	22.0	0.096	25.8	0.775	18.3	0.037	28.9	0.008
Bound residues, released via microwave hydrolysis with isopropanol, 0.5 N HCl, and/or 0.5 N NaOH:								
CGA-153433	1.8	0.008	2.7	0.081	3.7	0.007	--	--
OH-CGA-153433	1.6	0.007	2.6	0.078	4.4	0.009	--	--
Unknowns/unassigned/origin	8.3	0.036	22.7	0.682	16.6	0.035	--	--
Unanalyzed fractions	9.9	0.043	4.5	0.137	1.0	0.002	--	--
Total identified	38.1	0.167	39.2	1.177	31.3	0.064	7.3	0.002
Total characterized	40.2	0.175	53.0	1.594	35.9	0.074	28.9	0.008
Total extractable	87.9	0.385	106.5	3.202	84.1	0.171	34.6	0.010
Unextractable (PES)[1]	6.2	0.027	1.4	0.042	8.2	0.017	57.8	0.017
Accountability[2]	94.1	94.1	107.9	107.9	92.3	92.2	92.4	93.1

[1] Post-extraction solids: residues remaining after exhaustive extractions.

[2] Accountability from ppm = (Total extractable + Total unextractable)/(TRR from combustion analysis; see TABLE C.2.1) * 100.

C.3. Proposed Metabolic Profile

FIGURE C.3.1. Proposed Metabolic Profile of Cloquintocet-Mexyl in Wheat.

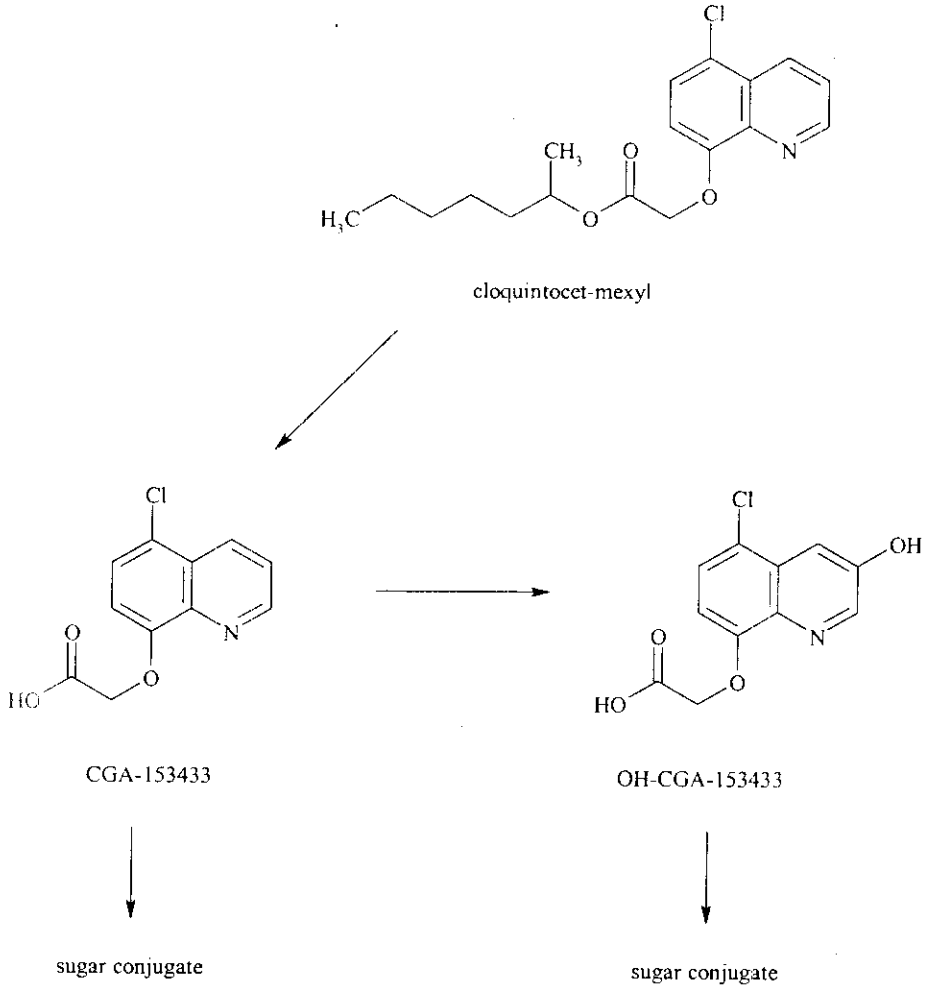
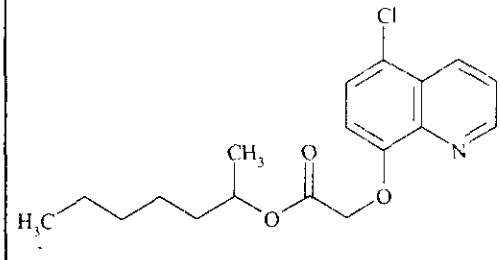
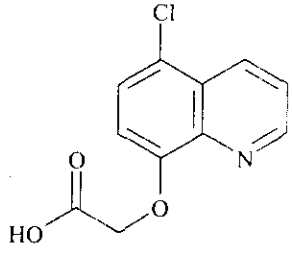
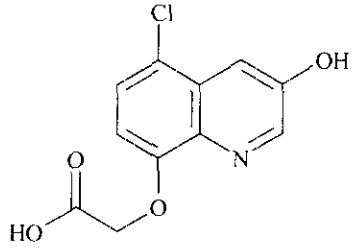


TABLE C.3.1. Identification of Compounds from Metabolism Study.		
Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
Cloquintocet-mexyl	1-methylhexyl [(5-chloro-8-quinolinyl)oxy]acetate	
CGA-153433	[(5-chloroquinolin-8-yl)oxy]acetic acid	
OH-CGA-153433	[(5-chloro-3-hydroxyquinolin-8-yl)oxy]acetic acid	

D. CONCLUSION

In wheat plants treated with [quinoline-3-^{14}C]cloquinotcet-mexyl at 0.016 lb safener/A, TRR were 0.438 ppm in forage harvested 7 days post-treatment, 0.019 ppm in forage harvested 30 days post-treatment, 0.004 ppm in mature straw, and 0.003 ppm in mature grain. In wheat plants treated with [quinoline-3-^{14}C]cloquinotcet-mexyl at 0.16 lb safener/A, TRR were 3.004 ppm in forage harvested 7 days post-treatment, 0.204 ppm in forage harvested 30 days post-treatment, 0.029 ppm in mature straw, and 0.012 ppm in mature grain.

Because of low radioactivity levels, samples of mature straw and grain from the low rate treatment were not subjected to extraction procedures. The majority of the TRR (~50-73%) was extracted from wheat forage using ACN/water; ACN/water released smaller amounts (29-35% TRR) from straw and grain. The petitioner subjected the ACN/water extract to cellulase hydrolysis and compared the chromatographic profile with that of the extract prior to hydrolysis. The nonextractable residues of 7-day PHI forage (both treatments) and 30-day PHI forage (high rate treatment), which accounted for 29-36% TRR, were subjected to sequential microwave

hydrolyses, using isopropanol/water, 0.5 N HCl, and 0.5 N NaOH to release additional residues. The nonextractable residues of 30-day PHI forage (low rate treatment) and mature straw and grain (high rate treatment) accounted for 0.006-0.017 ppm and were not subjected to additional characterization attempts. The accountabilities were 92-108%.

Approximately 31-39% TRR were identified in forage, and 7% TRR were identified in mature straw. Cloquintocet-mexyl was found in minor amounts (<4% TRR) in 7-day PHI forage only. The metabolite OH-CGA-153433 was found to be the major metabolite in forage (7-day PHI, both treatment rates, and 30-day PHI, high rate treatment) and in mature straw (high rate treatment): a large portion of these amounts in forage were conjugated OH-CGA-153433 (released after hydrolysis with cellulase). Metabolite CGA-153433 was also identified (<11% TRR) in forage; a portion of the amount identified in 7-day PHI forage (both treatment rates) was conjugated. Hydrolysis of bound residues yielded small amounts of CGA-153433 and OH-CGA-153433 (<5% TRR) in forage. A large portion of the remaining radioactivity consisted of unknowns; HPLC analyses of extracts and hydrolysates indicated that unknown peaks were each <5% TRR in 7-day PHI forage (both treatment rates), 30-day PHI forage (high rate treatment), and mature straw (high rate treatment).

Based on the results of this study, the petitioner concluded that metabolism of cloquintocet-mexyl is rapid, since low levels were observed in 7-day PHI forage and it was not detected in 30-day PHI forage or mature grain and straw. Cloquintocet-mexyl undergoes de-esterification to form CGA-153433 which is hydroxylated to form OH-CGA-153433. Further metabolism results in the binding of these metabolites to crop matrix and the generation of multiple polar components.

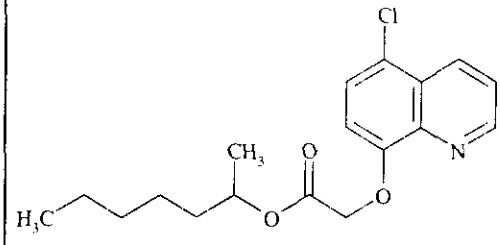
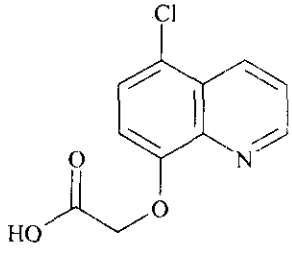
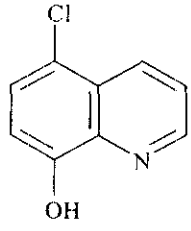
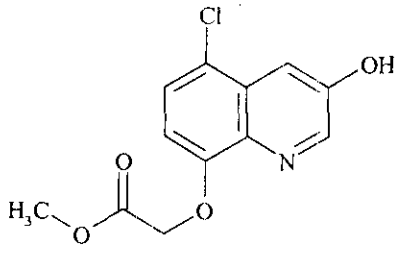
E. REFERENCES

DP Barcode: 257181
Subject: PP#7E04920. Cloquintocet-mexyl (Safener) in/on Wheat. Review of Analytical Methods and Residue Data. First Food Use Review.
From: Nancy Dodd
To: Bipin Gandhi/Robert Forrest
Dated: 4/7/00
MRIDs: 44387454, 44387457-44387461, 44399207-44399211, 44399213, 44399216-44399231, 44568401, 44568402, 44755301-44755303

F. DOCUMENT TRACKING

RDI: N. Dodd (11/16/05); W. Wassell (11/16/05)
Petition Number: 7E04920
DP Barcode: 308470
PC Code: 700099

Template Version September 2003

APPENDIX I. Chemical Names and Structures of Reference Standards Used in Spring Wheat Metabolism Study.		
Common name/code	Chemical name	Chemical structure
Cloquintocet-mexyl	1-methylhexyl [(5-chloro-8-quinolinyl)oxy]acetate	
CGA-153433	[(5-chloroquinolin-8-yl)oxy]acetic acid	
C-18469	5-chloroquinolin-8-ol	
CGA-151477	[(5-chloro-8-quinolinyl)oxy]acetic acid, methyl ester	

Primary Evaluator: Nancy Dodd, Chemist
Registration Action Branch 3
Health Effects Division (7509C)
Date: 11/16/05

Approved by: William Wassell, Chemist
Registration Action Branch 3
Health Effects Division (7509C)
Date: 11/16/05

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 12/15/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORTS:

46012910 Mair, P. (2003) Determination of Parent Compounds by HPLC. Lab Project Number: 871-00. Unpublished study submitted by Novartis Crop Protection, Inc. 21 p.

46012915 Pyles, S. and Hamilton, L. (2003) Radiovalidation of Analytical Method REM 138.12, "Determination of Parent Compounds by HPLC" in Wheat Forage, Fodder, and Grain. Lab Project Number: 223-01. Unpublished study submitted by Syngenta Crop Protection, Inc. 23 p.

EXECUTIVE SUMMARY:

Cloquintocet-mexyl was conditionally registered for use on wheat in June 2000 (PP#7E04920; DP Barcode D257181, 4/7/00, N. Dodd). In the previous review, HED concluded that the petitioner must submit a description of and radiovalidation data for Method REM 138.12. In response, Syngenta Crop Protection, Inc. has submitted a description of Method REM 138.12 for the determination of residues of cloquintocet-mexyl (parent) in/on wheat forage, grain, and fodder (straw). The petitioner has also submitted radiovalidation data for Method REM 138.12. Method REM 138.12 is a minor modification of Method REM 138.01. Method REM 138.12 is not proposed for enforcement.

Briefly, samples are extracted with acetonitrile (ACN), and fatty co-extractables are removed by partitioning with hexane. The extract is cleaned up by C-18 solid-phase extraction (SPE), followed by reextraction into hexane/diethyl ether and further cleanup by silica SPE. Cloquintocet-mexyl is eluted into separate fractions for analysis by high performance liquid chromatography with ultraviolet detection (HPLC/UV) with column switching. The reported limits of quantitation (LOQs) were 0.02 ppm for wheat grain and 0.05 ppm for wheat straw and forage, based on the lower limit of method validation. No validation data at the LOQ for wheat straw and forage were included in the current submission.

Method validation data for Method REM 138.12 demonstrated adequate method recoveries of cloquintocet-mexyl at the LOQ and 5x the LOQ for wheat grain, and at 2x the LOQ and 20x the

LOQ for wheat forage and straw. For cloquintocet-mexyl, recoveries were 72-91% (avg 81%) in wheat grain at fortifications of 0.02 and 0.4 ppm, 80-85% (avg 82%) in wheat straw at fortifications of 0.1 and 1.0 ppm, and 68-106% (avg 82%) in wheat forage at fortifications of 0.1 and 1.0 ppm.

The extraction efficiency data submitted for Method REM 138.12 indicate that the method extraction procedures do not release as much radioactivity as was released by the extraction procedures used in the metabolism studies.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the analytical method residue data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document DP Barcode 308470.

COMPLIANCE:

For MRID 46014910, signed and dated GLP and Data Confidentiality statements were provided. Because the submission is a description of a method, the GLP compliance statement pertained only to the method validation results presented with the method, and made reference to the studies in which the validation results were collected. Therefore, no Quality Assurance statement was included in the submission.

For MRID 46014915, signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported.

A. BACKGROUND INFORMATION

The safener cloquintocet-mexyl is included in systemic herbicide formulations to prevent damage to wheat plants from the phytotoxic effects of the herbicide. Discover® Herbicide/Discover NG® Herbicide, containing cloquintocet-mexyl and the active ingredient clodinafop-propargyl (CGA-184927), were conditionally registered for use on wheat in June 2000 (PP#7E04920; DP Barcode 257181, 4/7/00, N. Dodd). The petitioner has now submitted data to satisfy the conditions of full registration.

Syngenta has also petitioned for amended tolerances to support additional use of cloquintocet-mexyl as a safener in a pesticide formulation (Axial™ Herbicide) containing the new active ingredient pinoxaden for use on both wheat and barley. In connection with the pinoxaden petition, Syngenta proposed to revise the established tolerances for cloquintocet-mexyl residues in/on wheat commodities and proposed tolerances on barley commodities (PP#4E06831; 69 FR 31118, 6/2/04; and 69 FR 67731, 11/19/04).

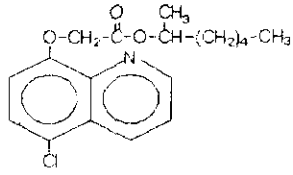
TABLE A.1. Test Compound Nomenclature	
Compound	Chemical Structure 
Common name	Cloquintocet-mexyl
Company experimental name	CGA-185072
IUPAC name	(5-chloroquinolin-8-yloxy)acetic acid 1-methylhexyl ester
CAS name	acetic acid, [(5-chloro-8-quinolinyl)oxy]-, 1-methylhexyl ester
CAS #	99607-70-2
End-use products/EPs	Discover® Herbicide (EPA Reg. No. 100-907) Discover® NG Herbicide (EPA Reg. No. 100-1173) Axial™ Herbicide (EPA Reg. No. 100-XXX)

TABLE A.2. Physicochemical Properties of the Technical Grade of Cloquintocet-mexyl		
Parameter	Value	Reference
Melting range	61.4 to 69°C	MRID 44387401
pH	5.4 at 25°C (1% w/v aqueous disp.)	MRID 44387401
Density	1.05 g/cm^3 at 22°C	MRID 44387401
Water solubility (25°C)	0.59 mg/L at pH 7.0 (PAI)*	MRID 44387401
Solvent solubility (g/L) (25°C)	ethanol - 190 acetone - 340 toluene - 360 n -hexane - 0.140 n-octanol - 11	MRID 44387401
Vapor pressure at 25°C	3.98 x10^{-8} mm Hg (PAI)*	MRID 44387401
Dissociation constant (pK_a)	3.55 (PAI)*	MRID 44387401
Octanol/water partition coefficient log P_{ow}	5.03 (at 25°C) (PAI)*	MRID 44387401
UV/visible absorption spectrum	Two absorbance maxima occur at 243.8-255.8 nm and 317.6-364.0 nm. No absorbance maxima occur between 370 nm and 750 nm.	MRID 44387401

*PAI = pure active ingredient

B. MATERIALS AND METHODS

B.1. Data-Gathering Method

B.1.1. Principle of the Method:

Method REM 138.12 determines residues of cloquintocet-mexyl (parent) in wheat grain, straw, and forage. Briefly, samples are extracted with acetonitrile (ACN), and fatty co-extractables are removed by partitioning with hexane. The extract is cleaned up by C-18 solid-phase extraction (SPE), followed by re-extraction into hexane/diethyl ether and further cleanup using silica SPE. Cloquintocet-mexyl is eluted into separate fractions for analysis by HPLC/UV with column switching.

TABLE B.1.1. Summary Parameters for the Analytical Method Used for the Quantitation of Cloquintocet-Mexyl Residues in Wheat Commodities.

Method ID	REM 138.12
Analyte	cloquintocet-mexyl
Extraction solvent/technique	Samples are homogenized and then extracted with ACN and filtered. The extract is diluted to volume with ACN.
Cleanup strategies	An aliquot of the extract is partitioned twice with hexane, and the hexane phases are discarded. The ACN phase is concentrated, mixed with water, and applied to a C-18 SPE cartridge; residues are eluted with acetone. The eluate is mixed with water and sodium chloride solution and partitioned twice with hexane:diethyl ether (9:1, v:v). The organic phases are combined and applied to a silica SPE cartridge; cloquintocet-mexyl residues are eluted with hexane:acetone (8:2, v:v) The eluate is concentrated. The cloquintocet-mexyl eluate is redissolved in n-hexane:ethanol (100:1.5 or 100:2, v:v).
Instrument/Detector	HPLC/UV with column switching, using Nucleosil-50 for the first column and Nucleosil-NH2 for the second column; isocratic mobile phases of n-hexane:ethanol (100:1.5, v:v, for the first column and 100:2, v:v, for the second column); UV detector at 244 nm.
Standardization method	Calibration curve of a minimum of four external standards.
Stability of std solutions	Not addressed in the submission.
Retention time	~10 minutes

B.2. Enforcement Method

The enforcement method for parent cloquintocet-mexyl is REM 186.01, which is discussed in DP Barcode 257181 (N. Dodd, 4/7/00) and in 46012912.de2.wpd.

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method

TABLE C.1.1. Recovery Results from Method Validation of Wheat Matrices using the Data-Gathering Analytical Method 138.12.[1]

Matrix	Analyte	Spiking Level (ppm)	Recoveries Obtained (%)	Mean Recovery ± SD [CV] (%)
Wheat grain	cloquintocet-mexyl	0.02	82, 85, 87, 91	81 ± 6.4 [7.9]
		0.4	72, 73, 77, 80, 85	
Wheat straw		0.1	80, 80, 84, 85	82 ± 2.2 [2.7]
		1.0	81, 81, 84, 85	
Wheat forage		0.1	68, 81	82 ± 17 [21]
		1.0	73, 106	

[1] Standards were prepared in ACN.

Confirmatory methods for Methods REM 138.01, REM 138.06, and REM 138.10 were submitted in MRID 46012911, which is discussed in the concurrent Residue Chemistry Summary Document DP Barcode 308470. The confirmatory method for Method REM 138.01 is applicable to Method REM 138.12 since the methods are similar. The identification of cloquintocet-mexyl may be confirmed by HPLC/MS/MS.

TABLE C.1.2. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of Cloquintocet-mexyl Residues in Wheat Commodities.

Analyte	cloquintocet-mexyl
Equipment ID	HPLC with Kratos UV/VIS detector 773; Nucleosil-50 and Nucleosil-NH2 columns; Valco C6W switching valve
Limit of quantitation (LOQ)	0.02 ppm for wheat grain and 0.05 ppm for wheat straw and forage, based on the lower limit of method validation. No validation data at the LOQ for wheat straw and forage were included in the current submission.
Limit of detection (LOD)	Not reported.
Accuracy/Precision	For cloquintocet-mexyl, recoveries were 72-91% (avg 81%) in wheat grain at fortifications of 0.02 and 0.4 ppm, 80-85% (avg 82%) in wheat straw at fortifications of 0.1 and 1.0 ppm, and 68-106% (avg 82%) in wheat forage at fortifications of 0.1 and 1.0 ppm, indicating acceptable accuracy/precision in the range of the spiking levels.
Reliability of the Method	No independent laboratory validation has been conducted for Method REM 138.12.
Linearity	No data pertaining to detector linearity were included in the submission.
Specificity	Peaks were well defined and symmetrical. There appeared to be no carryover to the following chromatograms.

The petitioner submitted radiovalidation data for Method REM 138.12 (MRID 46012915) using samples of wheat commodities from the wheat metabolism studies contained in MRIDs 46012908 and 46012909. The samples used for the study had been treated with [quinoline-3-^{14}C]cloquintocet-mexyl at 17.5 g safener/ha (0.016 lb safener/A) (forage) or 175 g safener/ha

(0.156 lb safener/A) (straw and grain). The petitioner noted that in the metabolism studies, no residues of cloquintocet-mexyl were found in mature grain and straw following treatment with [^{14}C]cloquintocet-mexyl. In wheat forage harvested 7 days following treatment with [^{14}C]cloquintocet-mexyl at 0.016 lb safener/A, residues of cloquintocet-mexyl were found at 0.015 ppm; however, this level is below the LOQ of Method 138.12.

To demonstrate extraction efficiency, the petitioner analyzed wheat forage (7-day PHI), grain, and straw samples from the metabolism studies using Method REM 138.12, as described above in Table B.1.1. The extracts were also analyzed by liquid scintillation counting (LSC) to determine radioactivity levels. The results of the extraction efficiency study are presented in Table C.1.4 below.

In conjunction with the extraction efficiency study, the petitioner additionally collected procedural recovery data (Table C.1.3 below) by fortifying untreated samples of wheat forage, straw, and grain with cloquintocet-mexyl at the LOQ (0.05 ppm for forage and straw and 0.02 ppm for grain).

TABLE C.1.3. Recovery Results from Method Validation of Wheat Matrices using the Data-Gathering Analytical Method 138.12 in Conjunction with the Radiovalidation Study.[1]

Matrix	Analyte	Spiking Level (ppm)	Recoveries Obtained (%)	Mean Recovery ± SD [CV] (%)
Wheat grain	cloquintocet-mexyl	0.02	153, 153, 154	153 ± 0.58 [0.38]
Wheat straw		0.05	76, 80, 90	82 ± 7.2 [8.8]
Wheat forage		0.05	135, 136, 139	137 ± 2.1 [1.5]

[1] Standards were prepared in ACN.

The petitioner noted that although there were samples with higher total radioactive residues generated in the metabolism studies, the samples chosen for the study were those with a sufficient amount of sample to allow triplicate analyses.

TABLE C.1.4. Extraction Efficiency of Method REM 138.12 Using Radiolabeled Samples from Wheat Metabolism Studies.

Matrix	Metabolism study	Residue Method [1]	Extraction Efficiency (%) [2]
Samples treated with [^{14}C]cloquintocet-mexyl at 0.016 lb safener/A			
Wheat forage, 7-day PHI			
TRR, ppm	0.438	Not determined[3]	--
%TRR extracted	63.9	20, 22, 23 (22)	34
Cloquintocet-mexyl, ppm	0.015	<0.05	Not applicable
Samples treated with [^{14}C]cloquintocet-mexyl at 0.156 lb safener/A			
Wheat straw			
TRR, ppm	0.029	Not determined	--
%TRR extracted	34.6	12, 14, 15 (14)	39
Cloquintocet-mexyl, ppm	None detected	<0.05	Not applicable
Wheat grain			
TRR, ppm	0.012	Not determined	--
%TRR extracted	29.0	2, 2, 3 (2.3)	8
Cloquintocet-mexyl, ppm	None detected	<0.02	Not applicable

[1] Average in parentheses.

[2] Extraction efficiency = (Average %TRR as determined by the residue method) ÷ (%TRR as determined in the metabolism study) x 100.

[3] Not determined: The level of residues determined in the metabolism study was used.

The submitted extraction efficiency data for Method REM 138.12 indicate that the method extraction procedures, in which samples are extracted with acetonitrile, do not release as much radioactivity as was released by the extraction procedures used in the metabolism studies, in which samples of wheat commodities treated with [^{14}C]cloquintocet-mexyl were extracted three times with ACN:water (8:2, v:v). In the metabolism studies, ACN/water extraction released 47-82% TRR from wheat forage samples and 15-38% TRR from wheat straw and grain samples. However, the metabolism study data indicated that no significant residues of cloquintocet-mexyl would be expected in wheat commodities.

C.2. Enforcement Method

The enforcement method for parent cloquintocet-mexyl is REM 186.01, which is discussed in DP Barcode 257181 (N. Dodd, 4/7/00) and in 46012912.de2.wpd.

C.3. Independent Laboratory Validation

No independent laboratory validation has been conducted for Method REM 138.12.

D. CONCLUSION

Adequate method validation data have been submitted for Method REM 138.12 for the determination of residues of parent cloquintocet-mexyl in wheat commodities.

The extraction efficiency data submitted for Method REM 138.12 indicate that the method extraction procedures do not release as much radioactivity as was released by the extraction procedures used in the metabolism studies.

E. REFERENCES

DP Barcode:	257181
Subject:	PP#7E04920. Cloquintocet-mexyl (Safener) in/on Wheat. Review of Analytical Methods and Residue Data. First Food Use Review.
From:	Nancy Dodd
To:	Bipin Gandhi/Robert Forrest
Dated:	4/7/00
MRIDs:	44387454, 44387457-44387461, 44399207-44399211, 44399213, 44399216-44399231, 44568401, 44568402, 44755301-44755303

F. DOCUMENT TRACKING

RDI: N. Dodd (11/16/05); W. Wassell (11/16/05)
Petition Number: 7E04920
DP Barcode: 308470
PC Code: 700099

Template Version September 2003

Primary Evaluator Nancy Dodd, Chemist Date: 11/16/05
Registration Action Branch 3
Health Effects Division (7509C)

Approved by William Wassell, Chemist Date: 11/16/05
Registration Action Branch 3
Health Effects Division (7509C)

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 12/15/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORTS:

46012912 Pyles, S. and Hamilton, L. (2003) Radiovalidation of Analytical Method REM 138.01, "Determination of Residues of Parent Compounds by Liquid Chromatography" in Wheat Fodder and Grain. Lab Project Number: 210-01. Unpublished study prepared by Syngenta Crop Protection, Inc. 24 p.

46012913 Pyles, S. and Hamilton, L. (2003) Radiovalidation of Analytical Method REM 138.10, "Determination of CGA-193469 and CGA-153433 By HPLC" in Wheat Forage, Fodder and Grain. Lab Project Number: 221-01. Unpublished study prepared by Syngenta Crop Protection, Inc. 23 p.

46012914 Pyles, S. and Hamilton, L. (2003) Radiovalidation of Analytical Method REM 138.06, "Determination of Residues of Metabolites CGA-153433 and CGA-193469 By Liquid Chromatography" in Wheat Fodder and Grain. Lab Project Number: 222-01. Unpublished study prepared by Syngenta Crop Protection, Inc. 22 p.

EXECUTIVE SUMMARY:

Cloquintocet-mexyl was conditionally registered for use on wheat in June 2000 (PP#7E04920; DP Barcode D257181, 4/7/00, N. Dodd). In the previous review, HED concluded that to satisfy the requirements of conditional registration, the petitioner must submit radiovalidation data for Methods REM 138.01, 138.06, and 138.10. Method REM 138.01 determines residues of cloquintocet-mexyl (parent) in wheat forage, straw, and grain. Methods REM 138.06 and 138.10 determine residues of the cloquintocet-mexyl metabolite CGA-153433 in wheat commodities.

Syngenta Crop Protection, Inc. has now submitted radiovalidation data for Methods REM 138.01, 138.06, and 138.10. The samples used for the study had been treated with [quinoline-3-^{14}C]cloquintocet-mexyl at 0.157 lb safener/A. The petitioner noted that in the metabolism

studies, no residues of cloquintocet-mexyl were found in mature grain and fodder (straw) following treatment with [^{14}C]cloquintocet-mexyl. In the samples used for radiovalidation, residues of CGA-153433 were identified in the metabolism studies at 0.010 ppm in 30-day PHI wheat forage. This level is below the limit of quantitation (LOQ) of Methods138.06 and 138.10 for wheat forage (0.05 ppm for forage).

The petitioner generated extraction efficiency data by subjecting wheat samples from the metabolism studies to the extraction procedures of Method REM 138.01 (grain and straw), Method REM 138.06 (grain and straw), and Method REM 138.10 (grain, forage, and straw). Based on the amount of radioactivity extracted, the extraction efficiency data indicate that Methods REM 138.06 and REM 138.10 would adequately extract incurred residues of CGA-153433 from wheat forage, straw, and grain samples. The extraction efficiency data submitted for Method REM 138.01 indicate that the method extraction procedures do not release as much radioactivity as was released by the extraction procedures used in the metabolism studies.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the analytical method residue data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document DP Barcode 308470.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported.

A. BACKGROUND INFORMATION

The safener cloquintocet-mexyl is included in systemic herbicide formulations to prevent damage to wheat plants from the phytotoxic effects of the herbicide. Discover® Herbicide/Discover NG® Herbicide, containing cloquintocet-mexyl and the active ingredient clodinafop-propargyl (CGA-184927), were conditionally registered for use on wheat in June 2000 (PP#7E04920; DP Barcode 257181, 4/7/00, N. Dodd). The petitioner has now submitted data to satisfy the conditions of full registration.

Syngenta has also petitioned for amended tolerances to support additional use of cloquintocet-mexyl as a safener in a pesticide formulation (Axial™ Herbicide) containing the new active ingredient pinoxaden for use on both wheat and barley. In connection with the pinoxaden petition, Syngenta proposed to revise the established tolerances for cloquintocet-mexyl residues in/on wheat commodities and proposed tolerances on barley commodities (PP#4E06831; 69 FR 31118, 6/2/04; and 69 FR 67731, 11/19/04).

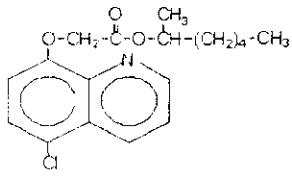
TABLE A.1. Test Compound Nomenclature	
Compound	Chemical Structure 
Common name	Cloquintocet-mexyl
Company experimental name	CGA-185072
IUPAC name	(5-chloroquinolin-8-yloxy)acetic acid 1-methylhexyl ester
CAS name	acetic acid, [(5-chloro-8-quinolinyl)oxy]-, 1-methylhexyl ester
CAS #	99607-70-2
End-use products/EPs	Discover® Herbicide (EPA Reg. No. 100-907) Discover® NG Herbicide (EPA Reg. No. 100-1173) Axial™ Herbicide (EPA Reg. No. 100-XXX)

TABLE A.2. Physicochemical Properties of the Technical Grade of Cloquintocet-mexyl		
Parameter	Value	Reference
Melting range	61.4 to 69°C	MRID 44387401
pH	5.4 at 25°C (1% w/v aqueous disp.)	MRID 44387401
Density	1.05 g/cm^3 at 22°C	MRID 44387401
Water solubility (25°C)	0.59 mg/L at pH 7.0 (PAI)*	MRID 44387401
Solvent solubility (g/L) (25°C)	ethanol - 190 acetone - 340 toluene - 360 n -hexane - 0.140 n-octanol - 11	MRID 44387401
Vapor pressure at 25°C	3.98 x10^{-8} mm Hg (PAI)*	MRID 44387401
Dissociation constant (pK$_a$)	3.55 (PAI)*	MRID 44387401
Octanol/water partition coefficient log P$_{ow}$	5.03 (at 25°C) (PAI)*	MRID 44387401
UV/visible absorption spectrum	Two absorbance maxima occur at 243.8-255.8 nm and 317.6-364.0 nm. No absorbance maxima occur between 370 nm and 750 nm.	MRID 44387401

*PAI = pure active ingredient

B. MATERIALS AND METHODS

B.1. Data-Gathering Method

B.1.1. Principle of the Method:

Method REM 138.01 determines residues of the safener cloquintocet-mexyl (parent) in wheat forage, straw, and grain, and Methods REM 138.06 and 138.10 determine residues of the cloquintocet-mexyl metabolite CGA-153433 in wheat commodities. The methods have been described and reviewed previously (DP Barcode 257181, 4/7/00, N. Dodd). Because the current submissions pertain only to radiovalidation data, no descriptions of the methods will be included in this review.

B.2. Enforcement Method

Methods REM 138.01 (for the parent cloquintocet-mexyl) and REM 138.10 (for the metabolite CGA-153433) are the current enforcement methods for plant commodities.

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method

The petitioner submitted radiovalidation data for Methods REM 138.01 (MRID 46012912), 138.06 (MRID 46012914), and 138.10 (MRID 46012913) using samples of wheat from the wheat metabolism studies contained in MRID 46012909. The samples used for the study had been treated with [quinoline-3-^{14}C]cloquintocet-mexyl at 0.157 lb safener/A. The petitioner noted that in the metabolism studies, no residues of cloquintocet-mexyl were found in mature grain and straw following treatment with [^{14}C]cloquintocet-mexyl. In the samples used for radiovalidation, residues of CGA-153433 were identified in the metabolism studies at 0.010 ppm in 30-day PHI wheat forage. This level is below the LOQ of Methods 138.06 and 138.10 for wheat forage (0.05 ppm for forage).

To demonstrate extraction efficiency, the petitioner subjected wheat samples from the metabolism studies to the extraction procedures of Method REM 138.01 (grain and straw), Method REM 138.06 (grain and straw), and Method REM 138.10 (grain, forage, and straw). The extracts were also analyzed by liquid scintillation counting (LSC) to determine radioactivity levels. The results of the extraction efficiency study are presented in Table C.1.2 below.

The petitioner additionally collected procedural recovery data by fortifying untreated samples of wheat commodities with cloquintocet-mexyl or CGA-153433. The results of the procedural recovery analyses are reported in Table C.1.1.

The petitioner noted that although there were samples with higher total radioactive residues generated in the metabolism studies, the samples chosen for the radiovalidation study were those with sufficient amount of sample to allow triplicate analyses.

TABLE C.1.1. Recovery Results from Method Validation of Methods REM 138.01, 138.06, and 138.10 Conducted in Conjunction with the Radiovalidation Studies.

Analyte	Matrix	Spiking Level (ppm)	Recoveries Obtained (%)	Mean Recovery ± SD [CV] (%)
Method REM 138.01				
Cloquintocet-mexyl	Wheat grain	0.02	101, 105, 126	102 ± 12 [12]
	Wheat straw	0.05	94, 94, 95	
Method REM 138.06				
CGA-153433	Wheat grain	0.05	70, 73, 75	73 ± 15 [21]
	Wheat straw	0.05	57, 61, 101	
Method REM 138.10				
CGA-153433	Recovery data for CGA-153433 were not submitted.			

TABLE C.1.2. Extraction Efficiency of Methods REM 138.01, 138.06, and 138.10 Using Radiolabeled Samples from Wheat Metabolism Studies.			
Matrix	Metabolism study	Residue Method	Extraction Efficiency (%) [1]
Method REM 138.01: Samples treated with [^{14}C]cloquintocet-mexyl at 0.157 lb safener/A			
Wheat straw			
TRR, ppm	0.029	Not determined[2]	--
%TRR extracted	34.6	4, 7, 11 (7)[3]	21
Cloquintocet-mexyl, ppm	None detected	<0.05	Not applicable
Wheat grain			
TRR, ppm	0.012	Not determined	--
%TRR extracted	29.0	2, 3, 3 (3)	9
Cloquintocet-mexyl, ppm	None detected	<0.02	Not applicable
Method REM 138.06: Samples treated with [^{14}C]cloquintocet-mexyl at 0.157 lb safener/A			
Wheat straw			
TRR, ppm	0.029	Not determined	--
%TRR extracted	34.6	29, 31, 31 (30)	88
CGA-153433, ppm	None detected	<0.05	Not applicable
Wheat grain			
TRR, ppm	0.012	Not determined	--
%TRR extracted	29.0	26, 27, 28 (27)	93
CGA-153433, ppm	None detected	<0.05 [4]	Not applicable
Method REM 138.10: Samples treated with [^{14}C]cloquintocet-mexyl at 0.157 lb safener/A			
Wheat forage, 30-day PHI			
TRR, ppm	0.204	Not determined	--
%TRR extracted	50.3	49, 49, 50 (49)	98
CGA-153433, ppm	0.010	Not determined	Not applicable
Wheat straw			
TRR, ppm	0.029	Not determined	--
%TRR extracted	34.6	31, 32, 32 (32)	92
CGA-153433, ppm	None detected	Not determined	Not applicable
Wheat grain			
TRR, ppm	0.012	Not determined	--
%TRR extracted	29.0	19, 20, 22 (20)	70
CGA-153433, ppm	None detected	Not determined	Not applicable

[1] Extraction efficiency = (Average %TRR as determined by the residue method) ÷ (%TRR as determined in the metabolism study) x 100.

[2] Not determined: The level of residues determined in the metabolism study was used.

[3] Average in parentheses.

[4] This value was reported by the petitioner in the data table; the method reports the LOQ to be 0.02 ppm for residues of CGA-153433 in/on grain.

The submitted radiovalidation data did not include analysis of any wheat forage samples using Method REM 138.01. However, the petitioner has separately submitted radiovalidation data for wheat forage samples using Method REM 138.12, which is very similar to Method REM 138.01; refer to the DER for MRID 46012915.

The submitted extraction efficiency data indicate that Methods REM 138.06 and REM 138.10 would adequately extract incurred residues of CGA-153433 from wheat forage, straw, and grain samples, based on the amount of radioactivity extracted. The extraction efficiency data submitted for Method REM 138.01 indicate that the method extraction procedures, in which samples are extracted with acetonitrile, do not release as much radioactivity as was released by the extraction procedures used in the metabolism studies, in which samples of wheat commodities treated with [^{14}C]cloquintocet-mexyl were extracted three times with ACN:water (8:2, v:v). In the metabolism studies, ACN/water extraction released 47-82% TRR from wheat forage samples and 15-38% TRR from wheat straw and grain samples. However, the metabolism study data indicate that no significant residues of cloquintocet-mexyl would be expected in wheat commodities.

C.2. Enforcement Method

Methods REM 138.01 and REM 138.10 are the current enforcement methods for plant commodities for cloquintocet-mexyl. Methods REM 138.01, 138.06, and 138.10 were forwarded to ACB/BEAD for petition method validation; ACB chose to conduct validation of Methods 138.01 and 138.10. The methods were adequately validated by ACB; however, minor modifications to the methods were required. Those modifications were listed in an EPA Addendum from ACB/BEAD. In MRID 45280101, Syngenta made changes to the methods REM 138.01 (Appendix 2) and REM 138.10 (Appendix 3) by reference to the EPA Addendum (Appendix 1). HED sent the revised methods to FDA for inclusion in PAM II as enforcement methods (DP Barcode 271447, 4/2/02, N. Dodd; memo to Mark Wirtz, FDA, from Nancy Dodd, 4/2/02).

C.3. Independent Laboratory Validation

Adequate independent laboratory validation data for Methods REM 138.01 and 138.06 have been reviewed previously (DP Barcode D257181, 4/7/00, N. Dodd). We note that Method REM 138.06 is very similar to Method REM 138.10.

D. CONCLUSION

The submitted data for Methods REM 138.01, 138.06, and 138.10 are adequate to satisfy residue analytical method radiovalidation data requirements. The extraction efficiency data submitted for Method REM 138.01 indicate that the method extraction procedures, in which samples are extracted with acetonitrile, do not release as much radioactivity as was released by the extraction

procedures used in the metabolism studies, in which samples of wheat commodities treated with [^{14}C]cloquintocet-mexyl were extracted three times with ACN:water (8:2, v:v).

E. REFERENCES

DP Barcode: 257181
Subject: PP#7E04920. Cloquintocet-mexyl (Safener) in/on Wheat. Review of Analytical Methods and Residue Data. First Food Use Review.
From: Nancy Dodd
To: Bipin Gandhi/Robert Forrest
Dated: 4/7/00
MRIDs: 44387454, 44387457-44387461, 44399207-44399211, 44399213, 44399216-44399231, 44568401, 44568402, 44755301-44755303

DP Barcode: 271447
Subject: PP#'s 7F04924 and 7E04920. Clodinafop-propargyl (CGA-184927) and Cloquintocet-mexyl (CGA-185072) on Wheat. Review of Revised Methods.
From: Nancy Dodd
To: Susan Stanton/Joanne Miller and Treva Alston/Kerry Leifer
Dated: 4/2/02
MRID: 45280101

F. DOCUMENT TRACKING

RDI: N. Dodd (11/16/05); W. Wassell (11/16/05)
Petition Number: 7E04920
DP Barcode: 308470
PC Code: 700099
Template Version September 2003

Primary Evaluator	Nancy Dodd, Chemist Date: 11/16/05 Registration Action Branch 3 Health Effects Division (7509C)
Approved by	William Wassell, Chemist Date: 11/16/05 Registration Action Branch 3 Health Effects Division (7509C)

STUDY REPORTS:

MRID 46203143. Crook, S.J. (2004) "Residue Method for the Determination of NOA 407854, SYN 505164, SYN 502836, SYN 505887 (Metabolites of NOA 407855), and CGA 153433 (Metabolite of CGA 185072) in Cereal Samples and Cereal Process Fractions. Final Determination by LC-MS/MS." Unpublished study prepared by Syngenta Crop Protection, Inc. Study No. REM 199.03. 74 pages. January 22, 2004.

MRID 46203142. Faltynski, K. H. (2004) Independent Laboratory Validation of Syngenta Method 117-01, "Analytical Method for Determination of NOA-407855, NOA-407854, SYN-505164, SYN-502836 and CGA-153433 in Crops by LC/MS/MS Including Validation Data." On Wheat (Forage, Straw, Grain and Aspirated Grain Fractions) and Barley (Hay and Grain). Unpublished study prepared by EN-CAS Analytical Laboratories. Study No. T001482-03. 129 pages. January 15, 2004.

MRID 46203139. Gasser, A. (2002) "Determination of NOA 407854, SYN 505164, SYN 502836, SYN 505887 (Metabolites of NOA 407855), and CGA 153433 (Metabolite of CGA 185072) in Cereals by LC/LC-MS/MS." Unpublished study prepared by Syngenta Crop Protection AG, Syngenta Crop Protection, Inc. Study No. REM 199.02. 59 pages. June 20, 2002.

MRID 46203140. Gasser, A. (2002) "Validation by Analysis of Wheat Specimens (Whole Plant, Straw and Grains) Fortified with NOA 407854, SYN 505164, SYN 502836, SYN 505887 and CGA 153433 and Determination of Recoveries." Unpublished study prepared by Syngenta Crop Protection AG, Syngenta Crop Protection, Inc. Study No. 02-S302. 52 pages. July 2, 2002.

MRID 46203138. Lin, K. (2003) "Analytical Method for Determination of NOA-407855, NOA-407854, SYN-505164, SYN-502836 and CGA-153433 in Crops by LC/MS/MS Including Validation Data." Unpublished study prepared by Dietary Safety Department of Syngenta Crop Protection, Inc. Study No. 117-01. 187 pages. December 5, 2003.

EXECUTIVE SUMMARY:

Syngenta submitted analytical methods REM 199.02, REM 199.03, and 117-01 for analysis of residues of CGA-153433, the metabolite of cloquintocet-mexyl, in cereal grain matrices. Method REM 199.02 was used to determine residues of CGA-153433 in barley grain, hay, and straw in one barley field trial study (MRID 46203205) and in wheat field trials conducted in Canada (MRID 46302206). Method 117-01 was used to determine residues of CGA-153433 in barley grain, hay, and straw in one barley field trial study (MRID 46203204) and in the barley grain and processed commodities in the processing study (MRID 46203204). (The methods also determine residues of pinoxaden in cereal matrices (DP Barcode 299651, Mohsen Sahafeyan, 7/7/05). All three methods possessed the same extraction procedure consisting of acid hydrolysis (1N HCl) by boiling under reflux for two hours.

Method REM 199.02 (MRID 46203139) was proposed as a data-gathering method. The method involved extracting homogenized crop samples with 1N HCl under reflux for 2 hours. Following filtration (if necessary), the pH of the extract was raised by addition of 3% ammonia solution and the solution was allowed to settle for half an hour. The analysis of the resulting extract was performed by reversed-phase high-performance liquid chromatography (HPLC) using a column-switching system connected via a pneumatically and thermally assisted electrospray ionization (ESI) to a tandem mass spectrometer (HPLC/HPLC-MS/MS). The limit of quantitation (LOQ) of the method for CGA-153433 was reported as 0.02 ppm in cereal whole plants, ears, stalks and straw and as 0.01 ppm in cereal grains. No limit of detection (LOD) was established. This method was found to give good recoveries within the acceptable range of 70-120% for the analysis of CGA-153433 in all the wheat matrices (whole plant, straw & grain) when spiked at the LOQ (0.01 ppm for grain and 0.02 ppm for all other matrices) and at 10X LOQ levels. The standard deviations (ranging from 1% to 12%) measured with respect to recoveries at each spiking level were indicative of the method having satisfactory repeatability. Good linearity was observed in the range of 0.35 to 20 ng/mL for CGA-153433 ($r^2 = 0.9996$). No independent laboratory validation (ILV) of this method was submitted. Based on the submitted recovery data for CGA-153433, analytical method REM 199.02 is acceptable as a data-gathering method for CGA-153433 in cereal matrices.

Method REM 199.03 (MRID 46203143) was proposed as a data-gathering method. The method involved extracting homogenized crop samples with 1N HCl under reflux for 2 hours. The pH of the extract was raised by addition of 3% ammonia solution (pH 3-4). The extract was centrifuged, filtered using a Vectaspin filtration tube and cleaned up on an Oasis HBL solid-phase extraction (SPE) cartridge eluted with dichloromethane:ethyl acetate:formic acid (80:20:0.5, v:v:v). The eluate was evaporated in the presence of 1N HCl solution and diluted with water prior to final analysis by HPLC-MS/MS. The LOQ of the method was reported as 0.02 ppm in cereal whole plants, ears, stalks and straw and as 0.01 ppm in cereal grains and cereal process fractions for CGA-153433. The LOD was estimated at 0.002 ppm for CGA-153433 in the matrices tested. Individual recoveries for CGA-153433 from Method REM 199.03 validation were all within the acceptable range of 70-120% for the analysis of barley whole plant

samples at spiking levels of 0.01 ppm (n=5 at each spiking level) and 0.1 ppm [TABLE C.1.1.]. Individual recoveries for barley grain samples spiked with CGA-153433 at levels of 0.01 ppm (LOQ) and 0.5 ppm were within 70-120% except for one value (68%). Recoveries for barley straw spiked with CGA-153433 at 0.01 ppm and 0.1 ppm were generally within 70-120% except for two values (58% and 62%). The standard deviations (ranging from 3% to 8%) measured with respect to recoveries at each spiking level were indicative of the method having satisfactory repeatability. Good linearity was observed in the range of 0.00125 to 0.5 µg/mL for CGA-153433; r^2 = 0.9993 (grain), 0.9998 (straw), and 0.9991 (whole plant). No ILV of this method was submitted. Based on the submitted recovery data, analytical method REM 199.03 is acceptable as a data-gathering method for CGA-153433 in cereal matrices.

Method REM 117-01 (MRID 46203138) is a proposed enforcement method. The method involved extracting homogenized crop samples with 1N HCl (or 1N HCl:acetonitrile (90:10, v:v)) under reflux for 2 hours. For determination of CGA-153433, an aliquot of the extract was filtered (if the solution was not clear) and after dilution with water, the final fraction was injected onto a reversed-phase C_{18} to ODS-3 two-column switching HPLC-MS/MS system for analysis. The C_{18} column was eluted with formic acid aqueous solution (0.1%):methanol (75:25, v:v) and the ODS-3 column with formic acid aqueous solution (0.05%):methanol (50:50, v:v). The LOQ of the method for CGA-153433 was reported as 0.02 ppm in cereal forage, hay and straw and as 0.01 ppm in cereal grains. The LOD of the method was reported as 0.00125 ng for CGA-153433. Syngenta Method 117-01 was found to give good recoveries within the acceptable range of 70-120% for the analysis of CGA-153433 in all the cereal matrices (grain, forage, hay & straw) when spiked at the LOQ (0.01 ppm for grain and 0.02 ppm for all other matrices) and up to ~1 ppm. The standard deviations (ranging from 2% to 10%) measured with respect to recoveries at each spiking level were indicative of the method having satisfactory repeatability. Good linearity was observed in the range of 0.00005 to 0.003 ng/µL for CGA-153433 (r^2 = 0.999889). The independent laboratory, EN-CAS Analytical Laboratories, successfully validated Method 117-01 in wheat (forage, straw, grain, and aspirated grain fractions) and barley (hay and grain) matrices. The method was able to extract CGA-153433 and quantitate it at the expected ranges. Based on the submitted recovery data for CGA-153433 and the ILV results, Syngenta Method 117-01 is acceptable as a data-gathering method for residues of CGA-153433 in cereal matrices. To be an enforcement method for cloquintocet-mexyl, EPA's analytical chemistry laboratory (ACB/BEAD) would have to validate the Method 117-01 for cloquintocet-mexyl (CGA-185072) and its metabolite CGA-153433 in cereal matrices and radiovalidation data for the method would have to be submitted.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the analytical methodology data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode 308470].

COMPLIANCE:

Reports REM 199.02 and REM 199.03 were not Good Laboratory Practice (GLP) studies; however, all the other study reports contained signed and dated GLP, Quality Assurance and Data Confidentiality statements. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode 308470].

A. BACKGROUND INFORMATION

The safener cloquintocet-mexyl is included in systemic herbicide formulations to prevent damage to wheat plants from the phytotoxic effects of the herbicide. Discover® Herbicide/Discover NG® Herbicide, containing cloquintocet-mexyl and the active ingredient clodinafop-propargyl (CGA-184927), were conditionally registered for use on wheat in June 2000 (PP#7E04920; DP Barcode 257181, 4/7/00, N. Dodd). The petitioner has now submitted data to satisfy the conditions of full registration.

Syngenta has also petitioned for amended tolerances to support additional use of cloquintocet-mexyl as a safener in a pesticide formulation (Axial™ Herbicide) containing the new active ingredient pinoxaden for use on both wheat and barley. In connection with the pinoxaden petition, Syngenta proposed to revise the established tolerances for cloquintocet-mexyl residues in/on wheat commodities and proposed tolerances on barley commodities (PP#4E06831; 69 FR 31118, 6/2/04; and 69 FR 67731, 11/19/04).

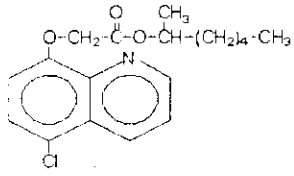
TABLE A.1. Test Compound Nomenclature	
Compound	Chemical Structure 
Common name	Cloquintocet-mexyl
Company experimental name	CGA-185072
IUPAC name	(5-chloroquinolin-8-yloxy)acetic acid 1-methylhexyl ester
CAS name	acetic acid, [(5-chloro-8-quinolinyl)oxy]-, 1-methylhexyl ester
CAS #	99607-70-2
End-use products/EPs	Discover® Herbicide (EPA Reg. No. 100-907) Discover® NG Herbicide (EPA Reg. No. 100-1173) Axial™ Herbicide (EPA Reg. No. 100-XXX)

TABLE A.2. Physicochemical Properties of the Technical Grade of Cloquintocet-mexyl		
Parameter	Value	Reference
Melting range	61.4 to 69°C	MRID 44387401
pH	5.4 at 25°C (1% w/v aqueous disp.)	MRID 44387401
Density	1.05 g/cm^3 at 22°C	MRID 44387401
Water solubility (25°C)	0.59 mg/L at pH 7.0 (PAI)*	MRID 44387401
Solvent solubility (g/L) (25°C)	ethanol - 190 acetone - 340 toluene - 360 n -hexane - 0.140 n-octanol - 11	MRID 44387401
Vapor pressure at 25°C	3.98 x10^{-8} mm Hg (PAI)*	MRID 44387401
Dissociation constant (pK$_a$)	3.55 (PAI)*	MRID 44387401
Octanol/water partition coefficient log P$_{ow}$	5.03 (at 25°C) (PAI)*	MRID 44387401
UV/visible absorption spectrum	Two absorbance maxima occur at 243.8-255.8 nm and 317.6-364.0 nm. No absorbance maxima occur between 370 nm and 750 nm.	MRID 44387401

*PAI = pure active ingredient

B. MATERIALS AND METHODS

B.1. Data-Gathering Methods

The three analytical methods proposed for the analysis of residues of CGA-153433 in plant matrices possessed the same extraction procedure, consisting of acid hydrolysis (1N HCl) by boiling under reflux for two hours. Under these conditions, cloquintocet-mexyl (parent) is converted to the metabolite CGA-153433. The methods thus analyze for parent as CGA-153433.

B.1.1.1. Principle of the Method: REM 199.02

Briefly, Method REM 199.02 involved extracting homogenized crop samples with 1N HCl under reflux for 2 hours. After cooling to room temperature, an aliquot was centrifuged and filtered (if necessary). The pH of the extract was raised by addition of 3% ammonia solution and the solution was let to settle for half an hour. The analysis of the resulting extract was performed by reversed-phase high performance liquid chromatography (HPLC) using a column-switching system connected via a pneumatically and thermally assisted electrospray ionization (ESI) to a tandem mass spectrometer (HPLC/HPLC-ESI/MS/MS). Retention times for CGA-153433 ranged from ~8.2-10.2 minutes.

The LOQ of the method for CGA-153433 was reported as 0.02 ppm in cereal whole plants, ears,

stalks and straw and as 0.01 ppm in cereal grains. No LOD was established.

The flow chart illustrating analytical method REM 199.02 is detailed in Appendix I.

TABLE B.1.1.1. Summary Parameters for the Analytical Method REM 199.02 Used for the Quantitation of CGA-153433 in Cereal Matrices.

Method ID	REM 199.02
Analyte	CGA-153433
Extraction solvent/technique	1N HCl under reflux for two hours.
Clean-up strategies	If solution is not clear, filter through a filter paper or a membrane filter (Acrodisc LC 13).
Instrument/Detector	HPLC/HPLC system with MS/MS detector, two pumps, a switching valve and an automatic sampling/ injection unit. HPLC/HPLC-ESI/MS/MS conditions: Analyte: CGA-153433 Volume Injected: 5 µL Pump 1: Shimatzu LC-10AD, isocratic flow rate: 0.25 mL/min Mobile Phase 1: HPLC-water:methanol:formic acid (800:200:2, v:v:v) Column 1: Xterra RP18, 50 x 2.1 mm i.d., 3.5 µm (oven temp. 40 °C) Switching Time: ~ 2 - 4 minutes Pump 2: Shimatzu LC-10AD, isocratic flow rate: 0.25 mL/min Mobile Phase 2: HPLC-water + methanol + formic acid (550 + 450 + 2 (v + v + v) Column 2: Inertsil ODS 3, 150 x 2.1 mm i.d., 3 µm (oven temp. 40 °C) Detector Instrument: API 4000 Triple Stage Quadrupole MS, atmospheric pressure ionization (API) tandem mass spectrometer Interface: TurbolonSpray (thermically and pneumatically assisted electrospray, ESI) Polarity Mode: Positive Selected Ions CGA-153433 Parent ion: m/z 238.0 (M+H)+ Daughter ion: m/z192.0
Standardization method	An external standard method was used as a marker for retention times, response and calibration.
Stability of std solutions	See Method REM 199.03.
Retention times	CGA-153433: approximately 8.2-10.2 minutes

B.1.1.2. Principle of the Method: REM 199.03

The following revision was made to Method REM 199.02:
Method REM 199.03 changed from column switching procedure to off-line SPE using single column final determination.

Briefly, Method REM 199.03 involved extracting homogenized crop samples with 1N HCl under reflux for 2 hours. After cooling to room temperature, an aliquot was taken, the pH of the extract was raised by addition of 3% ammonia solution (pH 3-4). The extract was centrifuged and

filtered using a Vectaspin filtration tube. Following centrifugation, the extract was cleaned up on an Oasis HBL solid-phase extraction (SPE) cartridge eluted with dichloromethane:ethyl acetate:formic acid (80:20:0.5, v:v:v). The eluate was evaporated in the presence of 1N HCl solution and diluted with water prior to final analysis by high performance liquid chromatography with triple quadrupole mass spectrometric detection (HPLC-MS/MS). Calibration was made using matrix matched standards. Retention time for CGA-153433 was 3.1 minutes. The LOQ of the method for CGA-153433 was reported as 0.02 ppm in cereal whole plants, ears, stalks and straw and as 0.01 ppm in cereal grains and cereal process fractions. The LOD was estimated at 0.002 ppm for CGA-153433 in the matrices tested.

TABLE B.1.1.2. Summary Parameters for the Analytical Method REM 199.03 Used for the Quantitation of CGA-153433 in Cereal Matrices.

Method ID	REM 199.03
Analyte	CGA-153433
Extraction solvent/technique	1N HCl under reflux for two hours.
Clean-up strategies	Filtration using a Vectaspin filtration tube Oasis HLB solid-phase extraction (SPE) clean-up [elution with dichloromethane:ethyl acetate:formic acid (80:20:0.5, v:v:v)].
Instrument/Detector	HPLC-MS/MS: Agilent 1100 series quaternary pump model number G1311A CGA-153433 is analyzed in the positive ionisation mode. HPLC-MS/MS conditions: Column: Ultracarb ODS (30) 50 mm x 4.6 mm i.d. or Ultracarb ODS (30) 50 mm x 3.2 mm i.d. Oven temperature: 40 °C Flow rate: 1.2 mL/min (4.6 mm i.d. column) 0.8 mL/min (3.2 mm i.d. column) Injection volume: 30 µL (CGA-153433) Mobile phase: A: Methanol B: 0.2% (v/v) formic acid in UP water

Instrument/Detector (Continued)	Gradient: **Mobile Phase Gradient (NOA 407854, SYN 505164, SYN 505887 and CGA 153433) 4.6 mm i.d. Column, Flow Rate 1.2 mL min^{-1}** Time (min) % A % B 0.0 20.0 80.0 4.0 90.0 10.0 4.5 90.0 10.0 4.6 20.0 80.0 5.5 20.0 80.0 **Mobile Phase Gradient (NOA 407854, SYN 505164, SYN 505887 and CGA 153433) 3.2 mm i.d. Column, Flow Rate 0.8 mL min^{-1}** Time (min) % A % B 0.0 20.0 80.0 4.0 90.0 10.0 5.0 90.0 10.0 5.1 20.0 80.0 6.0 20.0 80.0 **Mobile Phase Gradient (SYN 502836) 4.6 mm i.d. (Flow rate 1.2 mL min^{-1}) and 3.2 mm i.d. (Flow Rate 0.8 mL min^{-1}) Columns** Time (min) % A % B 0.0 20.0 80.0 4.0 90.0 10.0 4.1 20.0 80.0 5.0 20.0 80.0 <u>Detector</u> Instrument: Applied Biosystems API 3000 triple quadrupole mass spectrometer Interface: TurboIonSpray Scan type: Multiple Reaction Monitoring (MRM) Polarity: Positive <u>Selected Ions</u> CGA-153433 Parent ion: m/z 238.07 Daughter ion: m/z 178.95
Standardization method	An external standard method was used as a marker for retention times, response and calibration.
Stability of std solutions	In Method REM 199.03, the registrant stated: "When not in use, always store the standard solutions in a refrigerator at < 7 °C to prevent decomposition and/or concentration of the standard. It is recommended that analytical standards should be replaced with freshly prepared standards after four months." Furthermore, the registrant reported that: "Stability of the residues in the primary acid extracts was demonstrated for grain, straw and whole plant. Residues were stable for up to 14 days storage at 7 °C in grain and whole plant. Residues in straw gradually declined over 14 days, falling to below 50% for some analytes. Stability of the residues in the final solutions in vials was demonstrated in grain and straw. No decrease in residues was demonstrated over 7 days storage at 7 °C."
Retention times	CGA-153433: 3.1 minutes

B.1.1.3. Principle of the Method: 117-01

Briefly, Method REM 117-01 involved extracting homogenized crop samples with 1N HCl (or 1N HCl:acetonitrile (90:10, v:v)) under reflux for 2 hours. After cooling to room temperature, an aliquot was taken. For determination of CGA-153433, the aliquot was filtered (through an Acrodisc LC 13 mm filter) if the solution was not clear. Optional step: addition of concentrated ammonia to render the solution less acidic. After dilution with water, the final fraction was injected onto a reversed-phase C_{18} to ODS-3 two-column switching HPLC-MS/MS system for analysis. The C_{18} column was eluted with formic acid aqueous solution (0.1%): methanol (75:25, v:v) and the ODS-3 column with formic acid aqueous solution (0.05%): methanol (50:50, v:v).

TABLE B.1.1.3. Summary Parameters for the Analytical Enforcement Method 117-01 Used for the Quantitation of CGA-153433 in Plant Matrices.	
Method ID	117-01
Analyte	CGA-153433
Extraction solvent/technique	1N HCl (or 1N HCl:acetonitrile (90:10, v:v)) under reflux for two hours.
Clean-up strategies	For CGA-153433: The extract was cleaned up with a pre-conditioned SCX (2) SPE cartridge (elution with acetonitrile:water (25:75, v:v)) followed by a pre-conditioned C_8 SPE column (elution with acetonitrile:0.2% formic acid (50:50, v:v).
Instrument/Detector	High-Performance Liquid Chromatography (HPLC) with column-switching systems and triple quadrupole mass spectrometry (MS/MS) HPLC-MS/MS conditions: Analyte: **CGA-153433** Pump 1: Waters 2690 (Alliance) LC Pump with Autosampler Mobile Phase 1: formic acid aqueous solution (0.1%): methanol (75:25, v:v) Flow Rate 1: isocratic 0.25 mL/min Column 1: Xterra RP18, 50 x 2.1 mm i.d., 3.5 µm particle size Oven Temperature: 45 °C Injection Volume: 25 µL Switching Time: ~ 2 - 3 minutes and 8 - 10.5 minutes Pump 2: Perkin Elmer Model 250 Isocratic LC Pump Mobile Phase 2: formic acid aqueous solution (0.05%): methanol (50:50, v:v) Flow Rate 2: isocratic 0.25 mL/min Column 2: Metachem Technologies ODS-3, 150 x 2.1 mm i.d., 3 µm particle size Oven Temperature: 45 °C <u>Detector</u> Instrument: Micromass Quattro LC Positive Mode Parent ion: m/z 238 (M + H) + Daughter ion: m/z 179 (M + H) + Multiple Reaction Monitoring (MRM): m/z 238 ➜179
Standardization method	An external standard method was used as a marker for retention times, response and calibration.
Stability of std solutions	See Method REM 199.03.
Retention times	CGA-153433: ~16.23 minutes

B.2. Enforcement Method

Analytical Method 117-01 is a proposed enforcement method in plant matrices for the analysis of residues of cloquintocet-mexyl as its metabolite CGA-153433.

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method

TABLE C.1.1. Recovery Results from Method Validation of Cereal Matrices using Methods REM 199.02, REM 199.03 and Method 117-01. Standards were prepared in solvent. [1]

Matrix	Spiking Level (ppm)	Recoveries Obtained (%)	Mean Recovery ± SD [2] (%)
Method REM 199.02			
CGA-153433			
Wheat whole plant	0.02	102, 104, 101, 100, 105	102 ± 2
	0.20	95, 95, 95, 91, 91	93 ± 2
Wheat straw	0.02	80, 77, 82, 78, 81	80 ± 2
	0.20	74, 74, 73, 74, 73	74 ± 1
Wheat grain	0.01	99, 107, 116, 107, 83	102 ± 12
	0.10	94, 97, 100, 102, 96	98 ± 3
Method REM 199.03			
CGA-153433			
Barley Grain	0.01	77, 90, 88, 88, 74	83 ± 7
	0.5	68, 78, 74, 75, 81	75 ± 5
Barley Straw	0.01	78, 70, 74, 78, 71	74 ± 4
	0.1	76, 75, 58, 71, 62	68 ± 8
Barley Whole Plant	0.01	84, 82, 83, 89, 82	84 ± 3
	0.1	90, 82, 90, 84, 85	86 ± 4
Method 117-01			
CGA-153433			
Grain	0.01	87, 98, 74, 88, 99, 76	87 ± 10
	0.1	92	92
	1	95, 93	94
Forage	0.02	84, 86, 82, 97, 100, 99	91 ± 8.2
	0.1	99, 96	98
	1	103, 101	102
Hay	0.02	77, 77, 81, 78, 78, 82	79 ± 2.1
	0.1	90, 92	91
	1	96, 95	96

Matrix	Spiking Level (ppm)	Recoveries Obtained (%)	Mean Recovery ± SD [2] (%)
Straw	0.02	79, 81, 85, 82, 81, 85	82 ± 2.4
	0.1	80, 96	88
	1	76, 93	84

[1] In Methods REM 199.02 & 199.03, the CGA-153433 stock solutions were prepared in acetonitrile. The mixed fortification solutions (containing CGA-153433 and 4 pinoxaden analytes) were prepared by dilution of the stock solutions with 1N HCl. In Method 117-01, the stock solutions and the mixed fortification solutions were prepared in methanol.

[2] Standard deviation is calculated for n = 3 or more.

Method REM 199.02

Method REM 199.02 was found to give good recoveries [TABLE C.1.1.] within the acceptable range of 70-120% for the analysis of CGA-153433 in all the wheat matrices (whole plant, straw & grain) when spiked at the LOQ (0.01 ppm for grain and 0.02 ppm for all other matrices) and at 10X LOQ levels. The standard deviations (ranging from 1% to 12%) measured with respect to recoveries at each spiking level were indicative of the method having satisfactory repeatability. Based on the submitted recovery data, analytical method REM 199.02 is acceptable as a data-gathering method for CGA-153433 in cereal matrices.

Method REM 199.03

Individual recoveries for CGA-153433 from Method REM 199.03 validation were all within the acceptable range of 70-120% for the analysis of barley whole plant samples at spiking levels of 0.01 ppm (n=5 at each spiking level) and 0.1 ppm [TABLE C.1.1.]. Individual recoveries for barley grain samples spiked at levels of 0.01 ppm (LOQ) and of 0.5 ppm were within 70-120% except for one value (68%). Recoveries for barley straw spiked at 0.01 ppm and 0.1 ppm were generally within 70-120% except for two values (58% and 62%). However, the standard deviations (ranging from 3% to 8%) measured with respect to recoveries at each spiking level were indicative of the method having satisfactory repeatability. Based on the submitted recovery data, analytical method REM 199.03 is acceptable as a data-gathering method for CGA-153433 in cereal matrices.

Method 117-01

Method 117-01 was found to give good recoveries [TABLE C.1.1.] within the acceptable range of 70-120% for the analysis of CGA-153433 in all the cereal matrices (grain, forage, hay & straw) when spiked at the LOQ (0.01 ppm for grain and 0.02 ppm for all other matrices) up to ~1 ppm. The standard deviations (ranging from 2% to 10%) measured with respect to recoveries at each spiking level were indicative of the method having satisfactory repeatability. Based on the submitted recovery data, analytical method 117-01 is acceptable as a data-gathering method for CGA-153433 in cereal matrices.

All three methods have been shown to be specific to the target analyte through the use of a specific detector (HPLC-MS/MS).

TABLE C.1.2.1. Characteristics for the Data-Gathering Analytical Method REM 199.02 Used for the Quantitation of CGA-153433 Residues in Cereal Matrices.	
Analyte	CGA-153433
Equipment ID	HPLC system with MS/MS detector, two pumps, a switching valve and an automatic sampling/ injection unit (HPLC/HPLC-ESI/MS/MS). HPLC Pump1: Shimatzu LC-10AD, column Xterra RP18 Pump 2: Shimatzu LC-10AD, column Inertsil ODS 3 Detector: API 4000 Triple Stage Quadrupole MS, atmospheric pressure ionization (API) tandem mass spectrometer Interface: TurbolonSpray (thermically and pneumatically assisted electrospray, ESI)
Limit of quantitation (LOQ)	0.01 ppm for cereal grain 0.02 ppm for cereal whole plants, ears, stalks and straw
Limit of detection (LOD)	Not established.
Accuracy/Precision	Method REM 199.02 was found to give excellent recoveries within the acceptable range of 70-120% for the analysis of CGA-153433 in all the wheat matrices (whole plant, straw & grain) when spiked at the LOQ (0.01 ppm for grain and 0.02 ppm for all other matrices) and at 10X LOQ levels. The standard deviation (ranging from 1% to 12%) indicated acceptable accuracy/precision.
Reliability of the Method/ [ILV]	No independent laboratory method validation [ILV] was conducted for method REM 199.02.
Linearity	The method/detector response was linear within the range of 0.35 to 20 ng/mL. coefficients of determination: r^2 = 0.9996 for CGA-153433
Specificity	The control chromatograms generally have no peaks above the chromatographic background and the spiked sample chromatograms contain only the analyte peak of interest. Peaks were well defined and symmetrical.

TABLE C.1.2.2. Characteristics for the Data-Gathering Analytical Method REM 199.03 Used for the Quantitation of CGA-153433 Residues in Cereal Matrices.	
Analyte(s)	CGA-153433
Equipment ID	HPLC-MS/MS: Agilent 1100 series quaternary pump model number G1311A HPLC Column: Ultracarb ODS (30) 50 mm x 4.6 mm i.d. or Ultracarb ODS (30) 50 mm x 3.2 mm i.d. Detector: Applied Biosystems API 3000 triple quadrupole mass spectrometer Interface: TurbolonSpray
Limit of quantitation (LOQ)	0.01 ppm for cereal grain 0.02 ppm for cereal whole plants, ears, stalks and straw
Limit of detection (LOD)	Estimated at 0.002 ppm for all analytes.
Accuracy/Precision	Individual recoveries for CGA-153433 from Method REM 199.03 validation were all within the acceptable range of 70-120% for the analysis of barley whole plant samples at spiking levels of 0.01 ppm (n=5 at each spiking level) and 0.1 ppm. Individual recoveries for barley grain samples spiked at levels of 0.01 ppm (LOQ) and 0.5 ppm were within 70-120% except for one value (68%). Recoveries for barley straw spiked at 0.01 ppm and 0.1 ppm were generally within 70-120% except for two values (58% and 62%). However, the standard deviations (ranging from 3% to 8%) measured with respect to recoveries at each spiking level were indicative of the method having satisfactory repeatability.
Reliability of the Method/ [ILV]	No independent laboratory method validation [ILV] was conducted for method REM 199.03.
Linearity	The method/detector response was linear within the range of 0.00125 to 0.5 µg/mL. Coefficients of determination for CGA-153433: r^2 = 0.9993 in grain; 0.9998 in straw; and 0.9991 in whole plant.
Specificity	The controls were <0.01 ppm in grain, <0.02 ppm in straw, and <0.02 ppm in stalks and ears. Peaks were well defined and symmetrical.

TABLE C.1.2.3. Characteristics for the Data-Gathering Analytical Method 117-01 Used for the Quantitation of CGA-153433 Residues in Plant Matrices.	
Analyte	CGA-153433
Equipment ID	High-Performance Liquid Chromatography (HPLC) with column-switching systems and triple quadrupole mass spectrometry (MS/MS). **CGA-153433** Pump 1: Waters 2690 (Alliance) LC Pump with Autosampler Column 1: Xterra RP18, 50 x 2.1 mm i.d., 3.5 µm particle size Pump 2: Perkin Elmer Model 250 Isocratic LC Pump Column 2: Metachem Technologies ODS-3, 150 x 2.1 mm i.d., 3 µm particle size Detector: Micromass Quattro LC
Limit of quantitation (LOQ)	0.01 ppm for cereal grain 0.02 ppm for cereal forage, hay and straw
Limit of detection (LOD)	0.00125 ng for CGA-153433
Accuracy/Precision	Method 117-01 was found to give excellent recoveries within the acceptable range of 70-120% for the analysis of CGA-153433 in all the cereal matrices (grain, forage, hay & straw) when spiked at the LOQ (0.01 ppm for grain and 0.02 ppm for all other matrices) up to ~1 ppm. The standard deviation (ranging from 2% to 10%) indicated acceptable accuracy/precision.
Reliability of the Method/ [ILV]	An independent laboratory method validation [ILV], Study No. T001482-03 , was conducted to verify the reliability of method 117-01 for the determination of residues of CGA-153433 in cereal matrices. The values obtained are indicative that method 117-01 is reliable.
Linearity	The method/detector response was linear. Coefficients of determination: CGA-153433 r^2= 0.999889 within the range of 0.00005 to 0.003 ng/µL
Specificity	The control chromatograms generally have no peaks above the chromatographic background and the spiked sample chromatograms contain only the analyte peak of interest. Peaks were well defined and symmetrical.

C.2. Enforcement Method

Analytical Method 117-01 is a proposed enforcement method in plant matrices for the analysis of residues of cloquintocet-mexyl as its metabolite CGA-153433. This proposed enforcement method for plant matrices has been validated and subjected to independent laboratory validation.

C.3. Independent Laboratory Validation

An independent laboratory method validation (ILV) was conducted by EN-CAS Analytical Laboratories to verify the reliability and reproducibility of the LC-MS/MS residue analytical method 117-01 for the determination of residues of cloqintocet-mexyl as its metabolite CGA-153433 in wheat and barley matrices.

Untreated samples of wheat (forage, straw, grain and aspirated grain fractions) and barley (hay and grain) were obtained from Syngenta. Each analytical set consisted of one reagent blank, two control samples and four spiked samples; two spiked at the limit of quantitation (LOQ; 0.01 ppm

for grain and 0.02 ppm for all the other matrices) and two spiked at 10X LOQ.

Method 117-01 was performed as written with three minor modifications:
1) Evaporation under N_2 was substituted for rotary evaporation during the SPE clean-up step; and
2) The run time was extended from 30 minutes to 35 minutes.

A few minor problems were encountered during the ILV trials resulting in the rejection of trials. The Sponsor was contacted in order to clarify and discuss the probable cause of these difficulties and to suggest solutions.

Of note, using a volume of 200 mL (instead of 100 mL) of extraction solvent in Step 3.2.3 helped to avoid any potential charring problems. Also, better recoveries were obtained for grain samples (both wheat and barley) when using 100% 1N HCl acidic extraction rather that the mixture of HCl:ACN (90:10, v:v).

Recovery results are presented in TABLE C.3.1. EN-CAS Analytical Laboratories successfully validated Syngenta Method 117-01 in wheat (forage, straw, grain and aspirated grain fractions) and barley (hay and grain) matrices.

The independent laboratory recommended method clarifications and procedures to be considered for inclusion in Method 117-01:
1. Mention that aliquot filtration can be expedited by overnight refrigerated storage of the extraction solvent.

TABLE C.3.1. Recovery Results Obtained by an Independent Laboratory Validation of Method 117-01 for the Determination of CGA-153433 in Wheat and Barley Matrices.

Metabolite	Spiking Level (ppm)	Recoveries Obtained (%)	Mean Recovery (%)
Wheat Forage			
CGA-153433	0.02	105, 104	104
	0.2	106, 106	106
Wheat Straw			
CGA-153433	0.02	96, 110	103
	0.2	104, 100	102
Wheat Grain (First attempt)			
CGA-153433	0.01	120, 101	111
	0.1	105, 107	106
Wheat Grain (Second attempt)			
CGA-153433	0.01	78, 84	81
	0.1	83, 84	84
Wheat AGF			
CGA-153433	0.02	85, 83	84

Metabolite	Spiking Level (ppm)	Recoveries Obtained (%)	Mean Recovery (%)
	0.2	87, 92	90
Barley Hay			
CGA-153433	0.02	112, 113	112
	0.2	97, 101	99
Barley Grain			
CGA-153433	0.01	81, 79	80
	0.1	88, 87	88

D. CONCLUSION

Based on the submitted recovery data, analytical method REM 199.02 used for the quantitation of CGA-153433 is considered acceptable as a data-gathering method in cereal matrices.

Based on the submitted recovery data, analytical method REM 199.03 used for the quantitation of CGA-153433 is considered acceptable as a data-gathering method in cereal matrices.

Based on the submitted recovery data, analytical method 117-01 used for the quantitation of CGA-153433 is considered acceptable as a data-gathering method in cereal matrices. An independent laboratory confirmed the validity of Method 117-01.

All three methods have been shown to be highly specific to the target analyte through the use of tandem mass spectrometric detector (HPLC-MS/MS); therefore, neither a confirmatory method nor an interference study is required. The methods were able to extract CGA-153433 and quantitate it within the expected range.

Based on the submitted recovery data for CGA-153433 and the ILV results, Syngenta Method 117-01 would be considered acceptable as a data-gathering method for residues of parent (CGA-185072) and CGA-153433 determined as CGA-153433 in cereal matrices provided validation/recovery data for the parent CCA-185072 are also available. To be an enforcement method for cloquintocet-mexyl, EPA's analytical chemistry laboratory (ACB/BEAD) would have to validate the Method 117-01 for cloquintocet-mexyl (CGA-185072) and its metabolite CGA-153433 in cereal matrices and radiovalidation data for the method for CGA-185072 and CGA-153433 would have to be submitted.

E. REFERENCES

DP Barcode 299651. Pinoxaden in/on Wheat and Barley. PP#4F6817. Summary of Analytical Chemistry and Residue Data. Mohsen Sahafeyan, 7/7/2005.

F. DOCUMENT TRACKING

RDI: N. Dodd (11/16/05); W. Wassell (11/16/05)
Petition Numbers: 7E04920 and 4E06831
DP Number: 308470
PC Code: 700090
Template Version September 2003

APPENDIX I

FIGURE I.1 Flow Chart of Analytical Method REM 199.02

Weigh 10 g grain or 5 g whole plant, ear, stalk or straw specimen
into a 500 mL round bottom flask

↓

Fortify recovery specimens

↓

Add hydrochloric acid 1N to obtain a total extraction volume of 100 mL (taking into consideration the water content of the subspecimen as well as the volume of the fortification solution)

↓

Heat under reflux for two hours
Cool to room temperature and centrifuge an aliquot of the extract

↓

If necessary filter the supernatant solution

↓

Mix an aliquot of 6 mL of the clear solution with 3 mL of 3% ammonia solution

↓

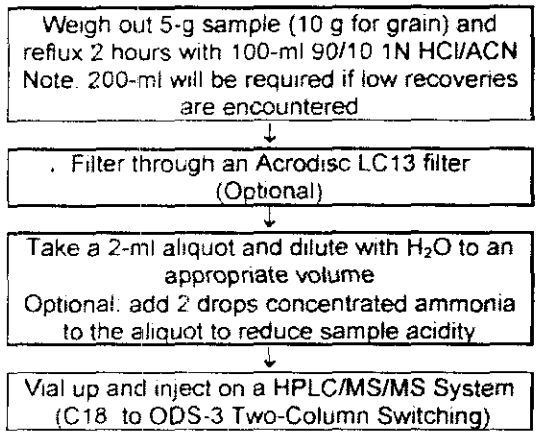
Let the solution settle for half an hour

↓

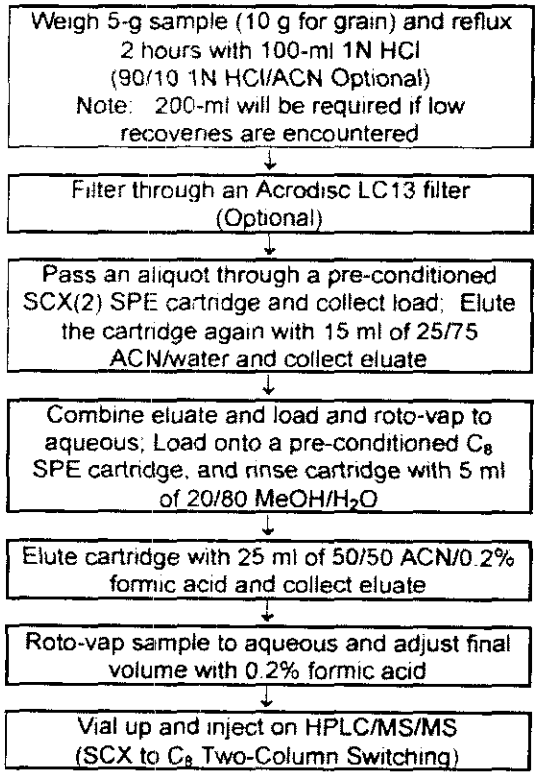
Inject the supernatant solution into the LC-LC-ESI/MS/MS system

FIGURE I.2 Flow Chart of Analytical Method 117-01

Determination of NOA-407855 and CGA-153433 (Fraction A)



Determination of SYN-505164 and SYN-502836 (Fraction B)



Primary Evaluator Nancy Dodd, Chemist Date: 11/16/05
Registration Action Branch 3
Health Effects Division (7509C)

Approved by William Wassell, Chemist Date: 11/16/05
Registration Action Branch 3
Health Effects Division (7509C)

This DER was originally prepared under contract by Toxicology and Hazard Assessment Group, Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830; submitted 4/15/05. The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

MRID 46373301. Close, C., Capps, T.M., Dixon III, M. (2003) [Quinolinyl-3-^{14}C]CGA-185072. Nature of the Residue in Lactating Goats. Syngenta Study Number 157-00. Unpublished study prepared by Syngenta Crop Protection, Inc., Greensboro, NC 138 pages.

EXECUTIVE SUMMARY:

[Quinolinyl-3-^{14}C]CGA-185072 (cloquintocet-mexyl, 99.6% radiochemical purity, 1.462 MBq/mg) was administered by gavage to two lactating Alpine goats once/day for 4 days at a concentration of ~4.3 mg/kg bw/day (126 ppm in the diet). Milk was collected twice daily, and urine, feces, and cage wash were collected daily at 24-hour intervals until sacrifice 6 hours after the last dose. Muscle, fat, liver, kidney and pooled bile were collected at sacrifice. The excreta, bile, milk, and tissue samples were extracted with acetonitrile and/or acetonitrile:water and analyzed chromatographically for radioactive components. Based on HPLC profiling of the extracts near the beginning and end of the study, the residues in the extracts were reasonably stable during the study.

Overall recovery of the radiolabel was adequate, with a mean recovery of 88% of the radioactivity administered to the two goats. Most of the radioactivity was excreted in the urine and feces. Mean recoveries from the two goats of the total dose administered were 0.2% of the dose in milk, ≤0.1% of the dose in each tissue (muscle, fat, kidney, and liver), 23% in the gastrointestinal (GI) tract, 52% in urine, and 12% in feces.

Tissue samples collected from the two goats six hours after the final dose contained mean total radioactive residues (TRR) of 0.017 ppm CGA-185072 equivalents in the leg muscle, 0.037 ppm in the fat, 0.214 ppm in the liver, and 2.907 ppm in the kidney. Mean residues in milk were 0.158-0.485 ppm (study avg 0.30 ppm) CGA-185072 equivalents.

The major metabolite recovered in the milk, tissues, bile, and excreta was CGA-153433, the ester hydrolysis product of CGA-185072. This metabolite accounted for 51.9% TRR in the liver,

53.4% TRR in the muscle, 67.1% TRR in the fat, 74.7% TRR in the kidney, and 80.2% TRR in the milk. Two other minor metabolites were identified. Small amounts of the metabolite M-2 were found in kidney, liver, muscle, and fat (≤5% TRR per matrix). Metabolite M-2 was formed through intramolecular cyclization, hydroxylation para to the nitrogen and reduction of the pyridine ring of CGA-185072. This metabolite was then conjugated to form the glucuronide metabolite known as M-1. Metabolite M-1 was found in kidney, liver, muscle, fat, and milk (≤11% TRR per matrix). Small amounts of parent were found in the kidney, liver, muscle, and fat (≤9.5%).

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the ruminant metabolism data are classified as scientifically acceptable.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document DP Barcode 308470.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would impact the validity of the study.

A. BACKGROUND INFORMATION

The safener cloquintocet-mexyl is included in systemic herbicide formulations to prevent damage to wheat plants from the phytotoxic effects of the herbicide. Discover® Herbicide/Discover NG® Herbicide, containing cloquintocet-mexyl and the active ingredient clodinafop-propargyl (CGA-184927), were conditionally registered for use on wheat in June 2000 (PP#7E04920; DP Barcode 257181, 4/7/00, N. Dodd). The petitioner has now submitted data to satisfy the conditions of full registration.

Syngenta has also petitioned for amended tolerances to support additional use of cloquintocet-mexyl as a safener in a pesticide formulation (Axial™ Herbicide) containing the new active ingredient pinoxaden for use on both wheat and barley. In connection with the pinoxaden petition, Syngenta proposed to revise the established tolerances for cloquintocet-mexyl residues in/on wheat commodities and proposed tolerances on barley commodities (PP#4E06831; 69 FR 31118, 6/2/04; and 69 FR 67731, 11/19/04).

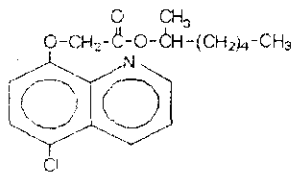
TABLE A.1. Test Compound Nomenclature	
Compound	Chemical Structure 
Common name	Cloquintocet-mexyl
Company experimental name	CGA-185072
IUPAC name	(5-chloroquinolin-8-yloxy)acetic acid 1-methylhexyl ester
CAS name	acetic acid, [(5-chloro-8-quinolinyl)oxy]-, 1-methylhexyl ester
CAS #	99607-70-2
End-use products/EPs	Discover® Herbicide (EPA Reg. No. 100-907) Discover® NG Herbicide (EPA Reg. No. 100-1173) Axial™ Herbicide (EPA Reg. No. 100-XXX)

TABLE A.2. Physicochemical Properties of the Technical Grade of Cloquintocet-mexyl		
Parameter	Value	Reference
Melting range	61.4 to 69°C	MRID 44387401
pH	5.4 at 25°C (1% w/v aqueous disp.)	MRID 44387401
Density	1.05 g/cm^3 at 22°C	MRID 44387401
Water solubility (25°C)	0.59 mg/L at pH 7.0 (PAI)*	MRID 44387401
Solvent solubility (g/L) (25°C)	ethanol - 190 acetone - 340 toluene - 360 n -hexane - 0.140 n-octanol - 11	MRID 44387401
Vapor pressure at 25°C	3.98 x10^{-8} mm Hg (PAI)*	MRID 44387401
Dissociation constant (pK_a)	3.55 (PAI)*	MRID 44387401
Octanol/water partition coefficient log P_{ow}	5.03 (at 25°C) (PAI)*	MRID 44387401
UV/visible absorption spectrum	Two absorbance maxima occur at 243.8-255.8 nm and 317.6-364.0 nm. No absorbance maxima occur between 370 nm and 750 nm.	MRID 44387401

*PAI = pure active ingredient

B. EXPERIMENTAL DESIGN

B.1. Livestock

TABLE B.1.1. General Test Animal Information

Species	Breed	Age	Weight at study initiation (kg)	Health Status	Description of housing/holding area
goat (Capra hircus)	Alpine	1.5 years	34.5	good	Housed individually in metabolism cages in a holding room with a temperature of 70-84°F, humidity of 26-69%, and a 12 hr light/dark cycle.
goat (Capra hircus)	Alpine	1.5 years	35.5	good	

TABLE B.1.2. Test Animal Dietary Regime

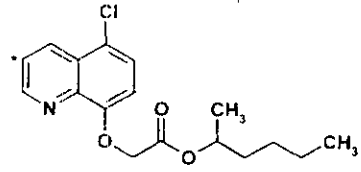
Composition of Diet	Feed consumption (kg/day)	Water	Acclimation period	Predosing
grain	1.4	Bottled water *ad libitum*	7 days in metabolism cages	None
hay	0.8			

TABLE B.1.3. Test Animal Dosing Regime

Treatment Type	Feeding Level (ppm test material in food)	Vehicle	Timing/Duration
Oral	126	capsule	0700 daily for four days

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics

Chemical structure	 * position of radiolabel
Radiolabel position	3 position of the quninoline ring
Lot No.	GAN-XLV-41-1
Purity	>98.6% as supplied by Syngenta Chemical Synthesis (method of determination not reported).
Specific activity (Bq)*	1.462 MBq/mg

*Bq = disintegrations per second

B.3. Sampling Information

TABLE B.3.1. Sample Collection Information			
Milk was collected ~0630 and ~1400 daily with an ~average 1400 g milk produced/goat/day.	Urine and feces were collected at 24 hour intervals from two days prior to dosing until sacrifice. Cage wash was not described.	Interval from last dose to sacrifice was ~ 6 hours.	Tissues harvested and analyzed were muscle (leg, tenderloin), fat (omental, perirenal), kidney, liver, bile, and gastrointestinal tract. The two muscle and fat samples were combined.

B.4. Identification/ Characterization of Residues

B.4.1. Sample Handling and Preparation

Liquid samples were stored refrigerated until completion of radioanalysis; solid and semi-solid samples were stored frozen. All samples were then shipped frozen from Vero Beach Research Center (VBRC), Vero Beach, FL, to Syngenta Crop Protection, Greensboro, NC where they remained frozen (at approximately -20°C) until analysis. Concentrated sample extracts were kept frozen (at an average temperature of -20°C) or refrigerated (at an average temperature of 4°C) between chromatographic analyses.

Total radioactive residues (TRR) in muscle, fat, liver, kidney, milk, urine, feces, blood, bile, and the GI tract were determined by combustion/liquid scintillation counting or directly by liquid scintillation counting. Composite samples of muscle, fat, liver, kidney, milk, urine, feces, and bile were also prepared and analyzed by combustion/liquid scintillation counting or directly by liquid scintillation counting.

All tissue and feces samples were homogenized in dry ice before extraction. Tissues, feces, and milk were extracted with acetonitrile and/or acetonitrile:water (8:2). The composite milk samples were extracted with acetonitrile (in a 1:1 ratio of acetonitrile:sample, v/w) for one hour. The extractions were centrifuged, the organic layer decanted, and the radioactivity determined in triplicate samples. The resulting pellet was extracted two additional times with 8:2 acetonitrile:water and the radioactivity determined. The radioactivity contained in the three extractions was combined to become the extractable residue, while the radioactivity contained in the pellet became the non-extractable residue.

Selected composite tissue samples were extracted under vacuum by using pre-packed C18 phase extraction columns. The columns were preconditioned with methanol. The samples, solubilized in 8:2 acetonitrile:water, were loaded onto the column and rinsed with 8:2 acetonitrile: water. The columns were eluted with 100% acetonitrile followed by chloroform with each eluant collected individually. Each eluant was analyzed for radioactivity and fractions containing ^{14}C were concentrated and characterized by chromatography. LOQ's (limits of quantitation) were not reported.

Some subsamples of post-extraction solids (PES) were air-dried and resuspended in 10 times the volume of pH 7 tris(hydroxymethyl) aminomethane-HCl buffer. To this, collagenase (512 units/mg PES) and protease (4 units/mg PES) were added and the suspensions placed onto an orbital shaker for ≥2 hours. After mixing, the suspensions were centrifuged and the supernatant removed and filtered. The amount of radioactivity was determined in the supernatant, and if present, was concentrated and characterized by high performance liquid chromatography (HPLC). The PES was air dried and oxidized to determine the residual amount of ^{14}C contained.

Some HPLC isolated fractions were air-dried and reconstituted with 4 mM phosphate buffer (pH 6.8) containing 100 U/mL β-glucuronidase. The isolate mixture was incubated at 37°C for 17 hours. With each hydrolysis, a positive and negative control were included. The positive control consisted of phenophthalein glucuronic acid which when treated with 0.53% potassium hydroxide, would turn pink indicating the hydrolysis of glucuronic acid by the enzyme. The negative control consisted of the same compound but was incubated in phosphate buffer that did not contain the enzyme. No change in color indicated the phenophthalein glucuronic acid was intact.

Other isolated fractions were concentrated to dryness and acetylated with a 9:1 acetic anhydride:pyridine solution for 12 hours. Some of the samples were further incubated at 45°C to complete acetylation. At completion of the acetylation reactions, the factions were dried and resolubilized in acetonitrile for further characterization and purification.

B.4.2. Analytical Methodology

One- or two-dimensional thin layer chromatography (TLC) was done on 20 x 20 cm 250 µ silica gel 60 F_{254} or Diol $F_{254}S$ glass plates. Appropriate developing solutions were used. The plates were visualized at 254 nm and recorded using a Camag Reprostar II equipped with a Kodak DC260 digital camera. CGA 185072 and anticipated metabolites standards were co-chromatographed one-dimensionally.

Sample purification and identification was done using high performance liquid chromatography (HPLC). The HPLCs were equipped with a variable UV/visible detector, a radioisotopic detector, and fraction collectors. The samples were eluted onto 5 µm Rx-C8, Phenyl, or SB-phenyl columns using various gradient systems composed of 140 mM ammonium phosphate (pH 6) and acetonitrile. The limit of quantitation (LOQ) was 0.005 ppm in tissue and milk samples. Other instrumentation for compound identification included quadrupole mass spectrometry (MS) and nuclear magnetic resonance (NMR).

C. RESULTS AND DISCUSSION

As shown in Table C.1, subsamples of the liver, kidney, muscle, fat, and milk were extracted within 46 days of sacrifice and profiled by HPLC within 2 days of extraction. Per OPPTS 860.1300, storage stability data for metabolism samples are not needed for samples stored frozen less than 6 months. Storage stability data are not needed for the metabolism RAC samples because they were

stored for less than 6 months before analysis. To evaluate the storage stability of the extracts, the stored frozen extracts of fat, muscle, liver, kidney, and milk, which were initially profiled by HPLC within 2 days of extraction, were profiled by HPLC a second time 714-730 days following the initial extraction (i.e., near the conclusion of the study). Comparison of the initial and final HPLC profiles of the extracts indicated that the profiles were similar, indicating that the extracts were reasonably stable when frozen for 714-730 days. These data indicate that the residues in the extracts were reasonably stable during the length of time of the study.

Total radioactive residues (TRR) in samples were determined by combustion/liquid scintillation counting or directly by liquid scintillation counting. The metabolic products were identified by one- and two-dimensional TLC, HPLC, quadrupole mass spectrometry (MS), and nuclear magnetic resonance (NMR). The LOQ was 0.005 ppm for tissues and milk.

Table C.2.1 shows the overall recovery of the radiolabel was adequate, with a mean recovery of 88% of the radioactivity administered to the two goats. Most of the radioactivity was excreted in the urine and feces. Mean recoveries from the two goats of the total dose administered were 0.2% of the dose in milk, ≤0.1% of the dose in each tissue (muscle, fat, kidney, and liver), 23% in the gastrointestinal (GI) tract, 52% in urine, and 12% in feces. Tissue samples collected from the two goats six hours after the final dose contained mean residues of 0.017 ppm CGA-185072 equivalents in the leg muscle, 0.037 ppm in the fat, 0.214 ppm in the liver, and 2.907 ppm in the kidney. Mean residues in milk were 0.158-0.485 ppm (study avg 0.30 ppm) CGA-185072 equivalents.

Metabolic profiles were done on composite samples of milk, muscle, liver, kidney, fat, feces, bile, and urine. Following extraction and analysis by HPLC, similar metabolic profiles were found for each matrix. Urine was chosen as the matrix for metabolite identification because it had the greatest amount of radioactivity and is a liquid.

As shown in Tables C.2.2.1, C.2.2.2, and C.2.3, the primary source of ^{14}C in the tissues, excreta, bile, and milk was associated with the ester-linkage hydrolysis product 5-chloro-8-quinolin-oxyacetic acid (CGA-153433). This metabolite was isolated by purifying a subsample of urine by reverse phase HPLC and identified by two-dimensional TLC and by HPLC. MS and NMR analysis confirmed that the metabolite was CGA-153433.

Metabolite M-1 was purified from urine subsamples by TLC and HPLC. The metabolite was identified by HPLC, MS, and NMR spectroscopy and shown to be a chlorinated aglycone product with attachment of glucuronide at the 7 position. Metabolite M-2 was purified from urine subsamples and isolated and identified by two-dimensional TLC and HPLC as the aglycone that is formed following hydrolysis of metabolite M-1 with β-glucuronidase. This metabolite was recovered primarily in the liver, kidney, excreta, muscle, and fat. The parent compound was isolated by HPLC and two-dimensional TLC primarily in the feces.

Of the small amount of radioactivity recovered in milk (0.2% of the dose), 95.5% was in the soluble fraction while 0.6% remained with the PES. The majority of the radiolabel recovered in the

soluble fraction, 80.2% TRR, was identified as CGA-153433. The second most abundant component (1.2% TRR) was not identified, although it had retention times similar to the parent compound. The other discrete entity found in milk (metabolite M-1), accounted for 0.8% of the TRR.

In the muscle, 0.04% of the dose was recovered with 90.2% of the TRR being soluble while 14.8% remained with the PES. The radiolabel in the PES was not further characterized since it accounted for <0.05 ppm. The major radiolabel component in the soluble fraction was identified as CGA-153433 (53.4% TRR). Other identified components in the muscle included the metabolite M-1 (8.9% TRR), parent compound (6.8% TRR), and metabolite M-2 (3.5% TRR).

Approximately 0.06% of the administered dose was recovered in the kidney with 95.5% associated with the soluble fraction and 5.6% associated with the PES 1. The major component associated with the soluble fraction was identified as CGA-153433. After enzyme hydrolysis of the PES 1 to release additional radioactivity, identified residues were CGA-153433 (74.7% TRR), parent compound (2.0% TRR), metabolite M-1 (5.4% TRR), and metabolite M-2 (2.4% TRR).

Approximately 0.02% of the administered dose was recovered in the liver with 75.1% TRR associated with the soluble fraction and 20.1% with the PES 1. CGA-153433 was the major residue. After enzyme hydrolysis of the PES 1 to release additional radioactivity, identified residues were CGA-153433 (51.9% TRR), parent compound (1.8% TRR), metabolite M-1 (8.8% TRR), and metabolite M-2 (2.2% TRR).

The fat contained <0.01% of the administered dose. Of this, 96.2% was associated with the soluble fraction. Identification of the soluble fraction showed 67.1% of the TRR was CGA 153433, 11.1% TRR was the M-1 metabolite, 4.8% TRR was the M-2 metabolite, and 9.5% was the parent compound.

Urine was the major route of elimination, accounting for 52% of the administered dose. The metabolite CGA-153433 accounted for 79.8% of the TRR while the metabolites M-1 and M-2 accounted for 12.2% and 5.5% of the TRR, respectively.

The bile accounted for 0.01% of the administered dose with 85.3% TRR associated with CGA-153433. The metabolite M-1 accounted for 8.3% of the TRR.

The feces accounted for 12% of the recovered radiolabel, with 75.7% associated with the soluble fraction and 34.1% associated with the PES. The metabolite CGA-153433 contributed 69.3% of the TRR in the soluble fraction with the parent compound contributing 0.8% TRR. The remaining identified radiolabel components consisted of metabolites M-1 and M-2, which represented 1.0% and 0.4% of the TRR, respectively.

The proposed metabolic pathway for CGA-185072 in lactating goats is shown in Appendix 1. The parent compound is rapidly hydrolyzed to CGA-153433 (5-chloro-8-quinolinoxyacetic acid),

the major residue recovered in the tissues, milk, and excreta. A minor pathway involves the formation of the metabolite M-2 through intramolecular cyclization, hydroxylation para to the nitrogen and reduction of the pyridine ring. This metabolite is then conjugated to form the glucuronide metabolite known as M-1.

C.1. Storage Stability

TABLE C.1. Summary of Storage Conditions

Matrix -RAC and Extracts	Storage Temp. (°C)	Actual Storage Duration (days)	Interval of Demonstrated Storage Stability (days)[1]
Fat	-20	40 + (1)[3] + (720)[2]	(720)[2]
Muscle	-20	32 + (2) + (724)	(724)
Liver	-20	32 + (2) + (724)	(724)
kidney	-20	26 + (1) + (730)	(730)
Milk	-20	46 + (2) + (714)	(714)

[1] Storage stability data are not needed for the RAC because the metabolism RAC samples were stored for less than 6 months before extraction; however, evidence of storage stability is needed for the extracts and has been provided for the extracts by HPLC profiling 1-2 days after extraction and again at the end of the study.
[2] Numbers in parenthesis apply to the extracts.

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1. Total Radioactive Residues (TRR) in Milk, Tissues, and Excreta from Two Goats (mean values) given Four Daily Doses of (3-^{14}C)Quinoline-labeled Cloquintocet-mexyl (CGA-185072) at the Rate of 126 ppm[1,2]

Matrix	Time (hours)[2]	% of Total Dose	ppm, [^{14}C]Cloquintocet-mexyl Equivalents
Urine	0-24	11.20	172.122
	24-48	17.14	169.799
	48-72	18.49	231.389
	72-78	5.40	246.577
		(Total 52.23)	
Feces	0-24	2.22	15.898
	24-48	4.22	29.828
	48-72	4.57	36.814
	72-78	1.28	31.498
		(Total 12.29)	
Muscle	78	0.04	0.017
Fat	78	0.005	0.037
Kidney	78	0.06	2.907
Liver	78	0.02	0.214
Milk	0-7	0.02	0.208
	7-24	0.02	0.158
	24-31	0.04	0.462
	31-48	0.02	0.172
	48-55	0.03	0.433
	55-72	0.02	0.162
	72-78	0.03	0.485
		(Total 0.18)	
GI tract[3]	78	22.81	14.493
Blood	78	0.06	0.161
Bile	78	0.01	4.354
% of Administered Dose		**87.70**	

[1] Data from Table 2, page 44, and Table 3, pages 135-137 of MRID 46373301.
[2] Tissue samples were taken at 78 hrs, which was 6 hours after the last dose.
[3] GI tract = gastrointestinal tract.

TABLE C.2.2.1. Distribution of Parent and the Metabolites in Goat Matrices (Composite Samples) when Dosed with (3-^{14}C)Quinoline-labeled Cloquintocet-mexyl (CGA-185072) [1]

Metabolite Fraction	Kidney 2.872 ppm		Liver 0.235 ppm		Muscle 0.018 ppm		Fat 0.028 ppm		Milk 0.489 ppm		Urine 210.482 ppm		Feces 33.508 ppm		Bile 3.967 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Extractable residues [2]	95.5	2.743	75.1	0.177	90.2	0.016	96.2	0.027	95.5	0.467	NA[3]	NA	75.7	25.366	NA	NA
HPLC applied	95.5	2.743	67.9	0.159	82.6	0.015	93.0	0.026	84.0	0.411	100	210.482	75.7	25.366	100	3.967
Elution time (minutes) [4]																
5-15	5.5	0.157	---	---	2.0	<0.001	---	---	0.2	<0.001	---	---	---	---		
15-19	0.5	0.015	---	---	---	---	---	---	---	---	---	---	---	---		
22-34	7.0	0.202	0.6 0.9 4.3	0.001 0.002 0.010	2.9	<0.001	---	---	---	---	---	---	0.5	0.166	0.3 0.9 1.7	0.011 0.036 0.066
31-40 (M-1)[2]	4.4	0.125	7.1	0.017	8.9	0.002	11.1	0.003	0.8	0.004	12.2	25.704	1.0	0.328	8.3	0.331
35-41	---	---	---	---	---	---	---	---	0.4 0.8	0.002 0.004	---	---	0.7	0.225	2.3	0.091
38-45 (CGA-153433)	72.0	2.066	48.1	0.113	53.4	0.010	67.1	0.019	80.2	0.392	79.8	167.954	69.3	23.236	85.3	3.383
42-46													2.2	0.737		
44-47 (M-2)	2.4	0.070	2.2	0.005	3.5	<0.001	4.8	0.001	---	---	5.5	11.484	0.4	0.136		
47-55 (CGA-185072)	2.0	0.056	1.8	0.004	6.8	0.001	9.5	0.003	---	---	---	---	0.8	0.267		
47-55 (Not CGA-185072)	1.4	0.041	0.4	<0.001	---	---	---	---	1.2	0.006	1.1 0.9	2.279 1.975	0.6	0.205	0.7	0.027
Not defined	0.3	0.010	2.5	0.006	5.1	<0.001	0.6	<0.001	0.4	0.002	0.5	1.086	0.2	0.067	0.6	0.022
PES 1 (post-extraction solids 1)	5.6	0.160	20.1	0.047	14.8	0.003	13.6	0.004	0.6	0.003	NA	NA	34.1	11.434	NA	NA

[1] Data are from Tables 3, 5, and 6 (pages 45 and 47-49) and pages 81-102 of MRID 46373301
[2] Tissues, feces, and milk were extracted with acetonitrile and/or acetonitrile:water (8:2).
[3] NA not applicable.
[4] Retention ranges vary slightly for different matrices; listed range encompasses all subset ranges.

TABLE C.2.2.2 Distribution of Parent and the Metabolites in Goat Matrices (Composite Samples) when Dosed with (3-^{14}C)Quinoline-labeled Cloquintocet-mexyl (CGA-185072): Enzyme Extraction of Kidney and Liver

Metabolite Fraction	Kidney 2.872 ppm		Liver 0.235 ppm	
	% TRR	ppm	% TRR	ppm
PES 1 (post-extraction solids 1)	5.6	0.160	20.1	0.047
Enzyme released	4.3	0.124	11.0	0.026
HPLC applied	4.0	0.115	10.3	0.024
Elution time (minutes)				
7-34	---	---	<0.001 <0.001 0.2	<0.001 <0.001 <0.001
26--34	0.3	0.007	2.3	0.005
34-40 (M-1)	1.0	0.028	1.7	0.004
36-41	--	---	1.2 0.7	0.003 0.002
40-45 (CGA-153433)	2.7	0.076	3.8	0.009
45-46 (M-2)	<0.1	0.002	---	---
Not defined	<0.1	0.001	0.4	<0.001
PES 2	1.6	0.047	7.2	0.017

Table C.2.3. Summary of Characterization and Identification of Radioactive Residues in Goat Matrices Following Application of (3-^{14}C)Quinoline-labeled Cloquintocet-mexyl (CGA-185072)

Compound	Kidney 2.872 ppm		Liver 0.235 ppm		Muscle 0.018 ppm		Fat 0.028 ppm		Milk 0.489 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Parent (CGA-185072)	2.0	0.056	1.8	0.004	6.8	0.001	9.5	0.003	ND [3]	---
M-1	4.4 1.0*	0.125 0.028	7.1 1.7*	0.017 0.004	8.9	0.002	11.1	0.003	0.8	0.004
CGA-153433	72.0 2.7*	2.066 0.076	48.1 3.8*	0.113 0.009	53.4	0.010	67.1	0.019	80.2	0.392
M-2	2.4 <0.1*	0.070 0.002	2.2	0.005	3.5	<0.001	4.8	0.001	ND	---
Unknowns/unassigned /origin	15.0	0.431	13.6	0.032	10.0	0.002	0.6	<0.001	3.0	0.015
Total identified	84.6	2.423	64.7	0.152	72.6	0.014	92.5	0.026	81.0	0.396
Total characterized	15.0	0.431	13.6	0.032	10.0	0.002	0.6	<0.001	3.0	0.015
Total extractable	99.8	2.866	86.1	0.202	90.2	0.016	96.2	0.027	95.5	0.467
Unextractable (PES) [1]	1.6	0.046	7.2	0.017	14.8	0.003	13.6	0.004	0.6	0.003
Accountability [2]	101.4	101.4	93.3	93.2	105.0	105.6	109.8	110.7	96.1	96.1

[1] Post-extraction solids: Residues remaining after exhaustive extractions.
[2] Accountability from ppm = (Total extractable + Total unextractable)/(TRRs from combustion analysis) * 100
Accountability (%) = % extractable - % unextractable.
[3] Not detected
* Enzyme-released residues.

D. CONCLUSION

Two lactating goats received oral doses of 126 ppm (~4.31 mg/kg/day) of [quinolinyl-3-^{14}C]CGA-185072 on four consecutive days and were sacrificed 6 hours following the final dose. Overall recovery of the radiolabel was adequate, with a mean recovery of 88% of the radioactivity administered to the two goats. Most of the radioactivity was excreted in the urine and feces. Mean recoveries from the two goats of the total dose administered were 0.2% of the dose in milk, ≤0.1% in each tissue (muscle, fat, kidney, and liver), 23% in the GI tract, 52% in urine, and 12% in feces.

Tissue samples collected from the two goats six hours after the final dose contained mean total radioactive residues (TRR) of 0.017 ppm CGA-185072 equivalents in the leg muscle, 0.037 ppm in the fat, 0.214 ppm in the liver, and 2.907 ppm in the kidney. Mean residues in milk were 0.158-0.485 ppm (study avg 0.30 ppm) CGA-185072 equivalents.

The major metabolite recovered in the milk, tissues, bile, and excreta was CGA-153433, the ester hydrolysis product of CGA-185072. This metabolite accounted for 51.9% TRR in the liver, 53.4% TRR in the muscle, 67.1% TRR in the fat, 74.7% TRR in the kidney, and 80.2% TRR in the milk. Two other minor metabolites were identified. Small amounts of the metabolite M-2 were found in kidney, liver, muscle, and fat (≤5% TRR per matrix). Metabolite M-2 was formed through intramolecular cyclization, hydroxylation para to the nitrogen, and reduction of the pyridine ring of CGA-185072 This metabolite was then conjugated to form the glucuronide metabolite known as M-1. Metabolite M-1 was found in kidney, liver, muscle, fat, and milk (≤11% TRR per matrix). Small amounts of parent were found in the kidney, liver, muscle, and fat (≤9.5% TRR). Parent was not detected in milk.

The proposed metabolic pathway for CGA-185072 in lactating goats is shown in Appendix 1. The parent compound is rapidly hydrolyzed to CGA-153433 (5-chloro-8-quinolinoxyacetic acid), the major residue recovered in the tissues, milk, and excreta. A minor pathway involves the formation of the metabolite M-2 through intramolecular cyclization, hydroxylation para to the nitrogen and reduction of the pyridine ring. This metabolite is then conjugated to form the glucuronide metabolite known as M-1.

E. REFERENCES

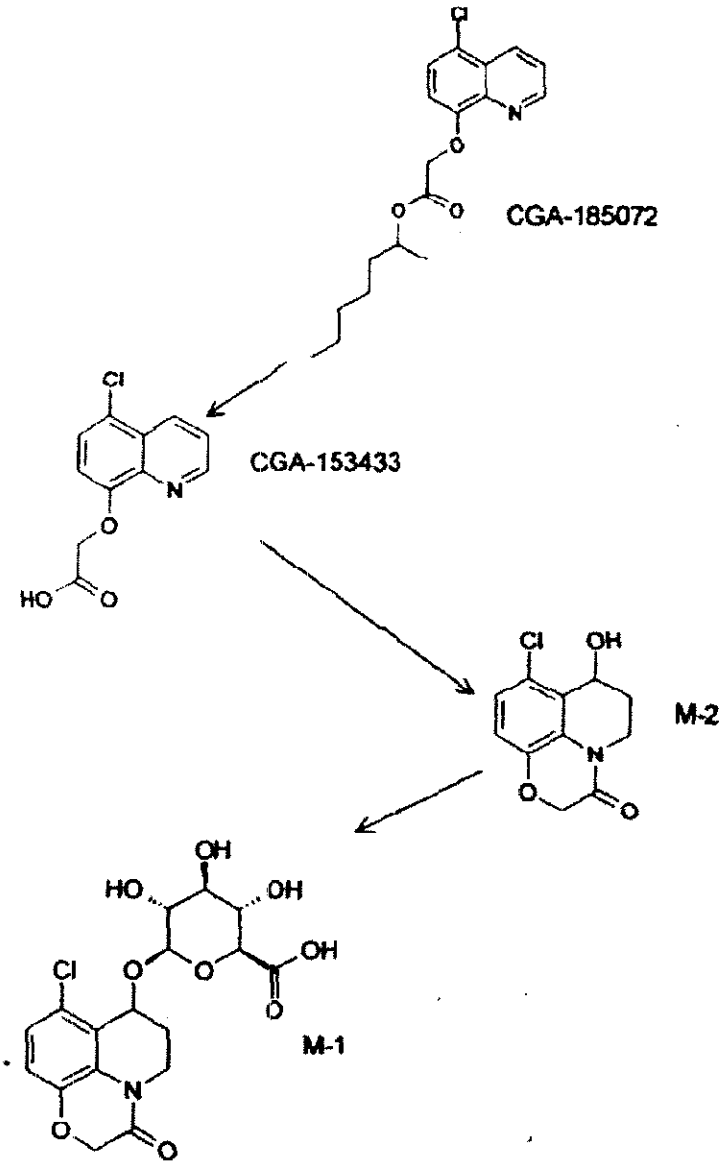
None

F. DOCUMENT TRACKING

RDI: N Dodd (11/16/05); W. Wassell (11/16/05)
Petition Number: 7E04920
DP Barcode: 308470
PC Code: 700099

APPENDIX 1

PROPOSED METABOLIC SCHEME FOR CGA-185072



Metabolic pathway of CGA-185072 in lactating goats

Primary Evaluator Nancy Dodd Date: 11/16/05
Registration Action Branch 3
Health Effects Division (7509C)

Approved by William Wassell, Chemist Date: 11/16/05
Registration Action Branch 3
Health Effects Division (7509C)

This DER was originally prepared under contract by Toxicology and Hazard Assessment Group, Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830; submitted 4/15/05. The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

MRID 46373302. Close, C., Fleischmann, T., Dixon III, M. (2003) [Quinolinyl-3-^{14}C]CGA-185072. Nature of the Residue in Layingns. Syngenta Study Number 158-00. Unpublished study prepared by Syngenta Crop Protection, Inc., Greensboro, NC. 91 pages.

EXECUTIVE SUMMARY:

[Quinolinyl-3-^{14}C]CGA-185072 (cloquintocet-mexyl, 99.6% radiochemical purity) was administered once per day orally to five white leghorn laying hens for 8 consecutive days at a feeding level of 7.4 mg/kg bw/day (95 ppm in the diet). Excreta and eggs were collected daily. Six hours after the final dose, the hens were sacrificed and the blood, skin with attached fat, thigh and breast muscle, peritoneal fat, and liver were collected. The residual carcasses were not examined. Muscle, liver, and egg yolks were extracted in acetonitrile/water (8:2). Egg white was extracted with acetonitrile. Fat was extracted with acetonitrile:hexane (2:1), by first stirring the fat in warm hexane and then adding the acetonitrile. Neutral solvent extractability was variable and ranged from 36% in the muscle to 87% in the fat. The excreta were not extracted. Enzyme hydrolysis of the muscle, liver, and egg yolk released most of the remaining radiolabeled residues. The neutral extracts were shown to be reasonably stable, based on high performance liquid chromatography (HPLC) profiling conducted 0-44 days after extraction and again 553-678 days after the initial profiling.

The majority of the dose (66%) was recovered in the excreta. Small amounts of the dose were recovered in tissues and eggs: 0.02% of the dose was recovered in the muscle and peritoneal fat, 0.04% was recovered in the liver, 0.01% was recovered in the egg white, and <0.01% was recovered in the egg yolk.

Total radioactive residues (TRR) were 0.010 ppm in muscle, 0.146 ppm in liver, 0.070 ppm in fat, 0.024-0.042 ppm in egg whites, and 0.002-0.018 ppm in egg yolks.

Essentially all of the radiolabel was associated with the ester hydrolysis product CGA-153433 (5-chloro-8-quinolinoxyacetic acid). This metabolite comprised 73.3% TRR in the fat, 64.9% TRR in the liver, 78.0% TRR in the egg white, 50.5% TRR in the egg yolk, and 50.5% TRR in the muscle. One other metabolic product was identified in the liver, that being the hydroxylated product of CGA-153433 (10.2% TRR).

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the poultry metabolism data are classified as scientifically acceptable.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document DP Barcode 308470.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would impact the validity of the study.

A. BACKGROUND INFORMATION

The safener cloquintocet-mexyl is included in systemic herbicide formulations to prevent damage to wheat plants from the phytotoxic effects of the herbicide. Discover® Herbicide/Discover NG® Herbicide, containing cloquintocet-mexyl and the active ingredient clodinafop-propargyl (CGA-184927), were conditionally registered for use on wheat in June 2000 (PP#7E04920; DP Barcode 257181, 4/7/00, N. Dodd). The petitioner has now submitted data to satisfy the conditions of full registration.

Syngenta has also petitioned for amended tolerances to support additional use of cloquintocet-mexyl as a safener in a pesticide formulation (Axial™ Herbicide) containing the new active ingredient pinoxaden for use on both wheat and barley. In connection with the pinoxaden petition, Syngenta proposed to revise the established tolerances for cloquintocet-mexyl residues in/on wheat commodities and proposed tolerances on barley commodities (PP#4E06831; 69 FR 31118, 6/2/04; and 69 FR 67731, 11/19/04).

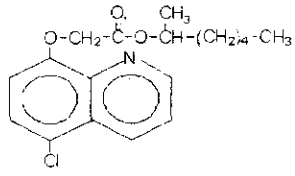
TABLE A.1. Test Compound Nomenclature	
Compound	Chemical Structure 
Common name	cloquintocet-mexyl
Company experimental name	CGA-185072
IUPAC name	(5-chloroquinolin-8-yloxy)acetic acid 1-methylhexyl ester
CAS name	acetic acid, [(5-chloro-8-quinolinyl)oxy]-, 1-methylhexyl ester
CAS #	99607-70-2
End-use products/EPs	Discover® Herbicide (EPA Reg. No. 100-907) Discover® NG Herbicide (EPA Reg. No. 100-1173) Axial™ Herbicide (EPA Reg. No. 100-XXX)

TABLE A.2. Physicochemical Properties of the Technical Grade Cloquintocet-mexyl		
Parameter	Value	Reference
Melting range	61.4 to 69°C	MRID 44387401
pH	5.4 at 25°C (1% w/v aqueous disp.)	MRID 44387401
Density	1.05 g/cm^3 at 22°C	MRID 44387401
Water solubility (25°C)	0.59 mg/L at pH 7.0 (PAI)*	MRID 44387401
Solvent solubility (g/L) (25°C)	ethanol - 190 acetone - 340 toluene - 360 n -hexane - 0.140 n-octanol - 11	MRID 44387401
Vapor pressure at 25°C	3.98 x10^{-8} mm Hg (PAI)*	MRID 44387401
Dissociation constant (pK_a)	3.55 (PAI)*	MRID 44387401
Octanol/water partition coefficient log P_{ow}	5.03 (at 25°C) (PAI)*	MRID 44387401
UV/visible absorption spectrum	Two absorbance maxima occur at 243.8-255.8 nm and 317.6-364.0 nm. No absorbance maxima occur between 370 nm and 750 nm.	MRID 44387401

*PAI = pure active ingredient

B. EXPERIMENTAL DESIGN

B.1. Livestock

TABLE B.1.1. General Test Animal Information

Species	Breed	Age	Weight at study initiation (kg)	Health Status	Description of housing/holding area
Laying hen	white leghorn (Gallus gallus domestica)	66 weeks	1.58-1.78	good	Hens are housed individually in 12 × 12× 18 inch metabolism cages in a holding room with a temperature of 65-88°F, humidity of 22-78%, and a 12- hr light/dark cycle.

TABLE B.1.2. Test Animal Dietary Regime

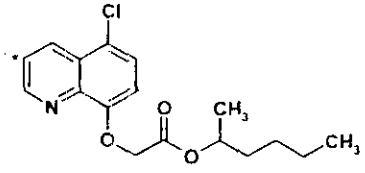
Composition of Diet	Feed consumption (kg/day)	Water	Acclimation period	Predosing
Layena® Crumbles (Purina Mills)	0.128-0.140	Bottled water *ad libitum*	7 days in metabolism cages	None

TABLE B.1.3. Test Animal Dosing Regime

Treatment Type	Feeding Level (ppm test material in food)	Vehicle	Timing/Duration
Oral	95	capsule	0700 daily for eight days

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics

Chemical structure	 * position of radiolabel
Radiolabel position	3 position on the quninoline ring
Lot No.	GAN-XLV-41-1
Purity	>98.6% As supplied by Syngenta Chemical Synthesis (method of determination not reported).
Specific activity (Bq)*	1.462 MBq/mg

*Bq = disintegrations per second

B.3. Sampling Information

TABLE B.3.1. Sample Collection Information			
Eggs were collected at ~0600-0700 hrs daily beginning 2 days before dosing until sacrifice. Average production = 4.5 eggs/day. After collection, eggs were separated into yolk and white.	Excreta was collected at ~0600-0700 hrs daily beginning 2 days before dosing until sacrifice. Cage wash was not described.	The interval from last dose to sacrifice was ~ 6 hours.	Tissues which were harvested and analyzed were blood, skin with attached fat, thigh and breast muscle, peritoneal fat, and liver. The carcass was collected but not analyzed.

B.4. Identification/ Characterization of Residues:

B.4.1. Sample Handling and Preparation

Liquid samples were stored refrigerated until completion of radioanalysis; solid and semi-solid samples were stored frozen. All samples were then shipped frozen from Vero Beach Research Center (VBRC), Vero Beach, FL, to Syngenta Crop Protection, Greensboro, NC, where they remained frozen (at approximately -20°C) until analysis. Concentrated sample extracts were kept frozen (at an average temperature of -20°C) or refrigerated (at an average temperature of 4°C) between chromatographic analyses.

Total radioactive residues (TRR) in muscle, fat, liver, egg yolks, egg whites, excreta, and blood were determined by combustion/liquid scintillation counting (LSC) or directly by liquid scintillation counting. Composite samples of muscle, peritoneal fat, liver, egg yolks, egg whites, and excreta were also prepared and analyzed by combustion/liquid scintillation counting or directly by liquid scintillation counting.

Subsample composite tissue and excreta were weighed and covered with sufficient acetonitrile:water (8:2) to be homogenized for five minutes. The homogenate was separated by centrifugation and the supernatant decanted into a graduated cylinder. The pellet was re-extracted at least one additional time and the supernatants combined and analyzed in triplicate. The nonextractable residues in the post-extraction solids (PES) were dried, weighed and homogenized; the TRR were determined by combustion analysis.

The egg whites were extracted three times using a magnetic stirrer and the yolks were extracted twice by homogenization in acetonitrile:water (8:2). The supernatants were recovered by centrifugation, combined, and reduced to dryness by rotary evaporation. They were reconstituted with acetonitrile before analysis. The PES were air dried and analyzed by combustion analysis.

The fat samples were extracted by producing a slurry in warm hexane and then adding acetonitrile while stirring. The slurry was centrifuged to separate the supernatant and re-

extracted a second time. Subsamples of the supernatant were analyzed by LSC. The PES was suspended in warm toluene before analysis by LSC.

Selected fat subsamples (0.1 g) were hydrolyzed for 7 days with lipase (0.5 g on days 1 and 2 and 0.1 g on day 4) suspended in TRIS-HCl buffer (pH = 7.5) at 25 parts buffer/lipase to sample (v:w). The samples were analyzed by LSC.

Selected PES samples were hydrolyzed with protease. A 1-1.5% protease solution in pH 7.0 TRIS-HCl buffer was added to the PES at ~ 5-20 parts buffer/enzyme to PES (v:w). The samples were incubated and placed on an orbital shaker for 1-2 days. They were then centrifuged and/or filtered and the supernatants analyzed by LSC and purified by C_{18}SPE columns before analysis by HPLC. The PES were analyzed by combustion analysis.

The fat samples were loaded onto C_{18}SPE columns that had been prewashed with 1% acetic acid in methanol and rinsed with 1% acetic acid to dry the column. The samples were loaded onto the column in 0.1% aqueous acetic acid and eluted with 0.1% acetic acid in methanol. Samples other than fat were purified on C_{18}SPE columns using neutral solvents for conditioning, loading, and eluting.

The control capsules were opened and individually placed in 250 mL volumetric flasks. Methanol was added to bring the contents to volume. Aliquots were removed for analysis by LSC.

B.4.2. Analytical Methodology

One or two dimensional thin layer chromatography (TLC) was done on 20 x 20 cm 250 µ silica gel 60 F_{254} glass plates. Appropriate developing solutions were used. The plates were visualized at 254 nm and recorded using a Camag Reprostar II equipped with a Kodak DC260 digital camera. CGA 185072 and anticipated metabolite standards were co-chromatographed one dimensionally.

Sample characterization was done using high performance liquid chromatography (HPLC). All HPLCs were equipped with a variable UV/visible detector, a radioisotopic detector, and a fraction collector. The samples were eluted onto 10 µm Whatman Partisil, 5 µm Zorbax C8, or 5 µm YMC Pack C8 columns using appropriate gradient solvents (these were composed of acetonitrile and water, 140 mM ammonium acetate (pH 6.0), or methanol). The limit of quantitation (LOQ) was 0.003 ppm in tissue and egg samples.

C. RESULTS AND DISCUSSION

As shown in Table C.1, tissue subsamples were extracted within 182 days of sacrifice. To determine the stability of residues in frozen extracts, subsamples of the original extracts of liver, egg yolk, egg white, muscle, and fat stored frozen at approximately -20°C were HPLC

profiled 0-44 days after extraction and HPLC profiled again 553-678 days after the initial profiling. Based on the results, the tissue and egg extracts were reasonably stable at freezer temperatures (approximately -20°C) during the length of the study.

Total radioactive residues (TRR) in samples were determined by combustion/liquid scintillation counting or directly by liquid scintillation counting. The metabolic products were identified by one- and two-dimensional TLC, HPLC, and liquid chromatography-mass spectrometry (LC-MS). The LOQ was 0.003 ppm for tissues and eggs.

As shown in Table C.2.1, total radioactive residues, expressed as cloquintocet-mexyl equivalents, were low in composite tissues and eggs, amounting to 0.010 ppm in muscle, 0.070 ppm in fat, 0.146 ppm in liver, 0.024-0.042 ppm in egg whites, and 0.002-0.018 ppm in egg yolks. The majority of the dose (66%) was recovered in the excreta. Small amounts of the dose were recovered in tissues and eggs: 0.02% of the dose was recovered in the muscle and peritoneal fat, 0.04% was recovered in the liver, 0.01% was recovered in the egg white, and <0.01% was recovered in the egg yolk. The percent of dose excreted in the feces remained relatively consistent through the 8-day study.

As shown in Table C.2.2, the neutral solvent extractability of muscle, fat, liver, and eggs was variable, ranging from 36% TRR in the muscle to 87% TRR in the fat. Enzyme hydrolysis released most of the remaining radiolabel. The excreta was not extracted.

The characterization/identification of the radiolabeled residues found in the tissues and eggs is summarized in Table C.2.3. The major residue was the ester hydrolysis product CGA-153433 (5-chloro-8-quinolinoxyacetic acid), accounting for 50.5% TRR in the muscle, 73.3% TRR in the fat, 64.9% TRR in the liver, 78.0% TRR in egg whites, and 50.5% TRR in egg yolks. Only one other metabolic product was identified and that was the hydroxylated product of CGA-153433. This metabolite was found only in the liver. No parent was found.

A metabolic scheme is provided in Appendix 1. CGA-185072 is hydrolyzed to the acid metabolite CGA-153433, which is the major residue. A small amount of further hydroxylation of the ring occurs to form an hydroxy-CGA-153433.

C.1. Storage Stability

TABLE C.1. Summary of Storage Conditions

Matrix - RAC and Extracts	Storage Temp. (°C) [1]	Actual Storage Duration (days)	Limit of Demonstrated Storage Stability (days) [2]
Muscle	-20	178 + (8)[3] + (622) [3]	(622) [3]
Fat	-20	182 + (44) + (553)	(553)
Liver	-20	175 + (2) + (657)	(657)
Egg White	-20	71 + (0) + (678)	(678)
Egg Yolk	-20	88 + (5) + (657)	(657)

[1] The average freezer temperature (from page 78 of MRID 46373302) is reported in the table.
[2] Storage stability data are not needed for the RAC samples because the metabolism RAC samples were stored for ≤6 months before extraction; however, evidence of storage stability is needed for the extracts and has been provided for the extracts by HPLC profiling 0-44 days after extraction and again at the end of the study.
[3] The numbers in parenthesis apply to the extracts.

TABLE C.2.1. Total Radioactive Residues (TRRs) in Eggs, Tissues, and Excreta from Five Hens (Composite Samples) Given Eight Daily Doses of (3-^{14}C)Quinoline-labeled Cloquintocet-mexyl (CGA-185072) at a Feeding Level of 95 ppm in the Diet.[1]

Matrix	Collection Timing (day)	% of Daily Dose	ppm, [^{14}C]Cloquintocet-mexyl Equivalents
Egg white	1	0.01	0.042
	2	0.01	0.039
	3	0.01	0.033
	4	0.01	0.033
	5	0.01	0.028
	6	0.01	0.033
	7	0.01	0.036
	8	0.00	0.024
		(avg. 0.01)	
Egg yolk	1	0.00	0.002
	2	0.00	0.005
	3	0.00	0.008
	4	0.00	0.012
	5	0.00	0.015
	6	0.00	0.016
	7	0.00	0.018
	8	0.00	0.018
		(avg 0.00)	
Excreta	1	72.95	107.738
	2	65.52	104.176
	3	67.26	113.010
	4	65.00	102.180
	5	59.42	79.335
	6	65.31	99.243
	7	68.83	103.029
	8	67.23	304.631
		(avg 66.44)	
Muscle	8	0.02	0.010
Liver	8	0.04	0.146
Fat	8	0.02	0.070
% of Administered Dose		**66.53**	--

[1] Data from Table 2, page 31, MRID 46373302.

TABLE C.2.2. Distribution of the Parent and Metabolites from Five Hens (Composite Samples) Given Eight Daily Doses of (3-^{14}C)Quinoline-labeled Cloquintocet-mexyl (CGA-185072) at a Feeding Level of 95 ppm in the Diet.[1]

Metabolite Fraction	Muscle 0.010 ppm		Fat 0.070 ppm		Liver 0.146 ppm		Egg white[2] 0.036 ppm		Egg Yolk[2] 0.018 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Solvent extractable[3]	35.5	0.004	86.6	0.061	82.9	0.121	83.1	0.030	54.6	0.010
HPLC Characterization										
Parent (CGA-185072)	ND[4]	ND	--	--	ND	ND	ND	ND	ND	ND
OH-CGA-153433	ND	ND	--	--	10.2	0.015	ND	ND	ND	ND
CGA-153433	31.2	0.003	--	--	64.9	0.095	78.0	0.028	37.8	0.007
unidentified minor metabolites	4.2	<0.001	--	--	7.8	0.011	5.2	0.002	16.8	0.003
PES 1 (post-extraction solids 1)	55.9	0.006	9.6	0.007	13.7	0.020	26.4	0.010	47.5	0.009
Enzyme released[5]	37.2	0.004	84.8	0.059	8.6[6]	0.013	19.1[6]	0.007	43.3	0.008
Parent (CGA-185072)	ND	ND							ND	ND
OH-CGA-153433	ND	ND							ND	ND
CGA-153433	19.3	0.002	73.3	0.051					12.7	0.002
unidentified minor metabolites	8.4	<0.001	9.0	0.006					8.1	0.001
PES 2 (post-extraction solids 2)	NR[7]	NR	NR	NR	1.1	0.002	1.1	<0.001	1.9	<0.001

[1] Data from Tables 2-4 on pages 31-33 and pages 45-58 of MRID 46373302.

[2] Egg whites and egg yolks were 7-day samples.

[3] Muscle, liver, and egg yolks were extracted in acetonitrile/water (8:2). Egg white was extracted with acetonitrile. Fat was extracted with acetonitrile:hexane (2:1), by first stirring the fat in warm hexane and then adding the acetonitrile.

[4] ND = not detected.

[5] The enzyme was protease except for fat. The extract from fat was hydrolyzed with lipase and centrifuged to produce a supernatant (84.8% TRR; 0.059 ppm) and more solids (an unreported amount) to add to the PES. The supernatant was analyzed by HPLC. The PES for fat were not subjected to protease hydrolysis.

[6] Protease-released solubles for liver and egg white were not further analyzed.

[7] Not reported.

Table C.2.3. Summary of Characterization and Identification of Radioactive Residues in Poultry Matrices (Composite Samples) from Five Hens Dosed for Eight Days with (3-^{14}C)Quinoline-labeled Cloquintocet-mexyl (CGA-185072) at a Feeding Level of 95 ppm in the Diet.

Compound	Muscle 0.010 ppm		Fat 0.070 ppm		Liver 0.146 ppm		Egg White 0.036 ppm		Egg Yolk 0.018 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Parent (CGA-185072)	---	ND [1]	---	ND	---	ND	---	ND	--	ND
OH-CGA-153433	---	ND	---	ND	10.2	0.015	---	ND	---	ND
CGA-153433	50.5	0.005	73.3	0.051	64.9	0.095	78.0	0.028	50.5	0.009
unidentified minor metabolites	12.6	0.001	9.0	0.006	7.8	0.011	5.2	0.002	24.9	0.004
Total identified	50.5	0.005	73.3	0.051	75.1	0.110	78.0	0.028	50.5	0.009
Total characterized	12.6	0.001	9.0	0.006	7.8	0.011	5.2	0.002	24.9	0.004
Total extractable	72.7	0.007	86.6	0.061	91.5	0.134	102.2	0.037	97.9	0.018
Unextractable (PES) [2]	NR [4]	NR	9.6	0.007	1.1	0.002	1.1	<0.001	1.9	<0.001
Accountability [3]	NA [5]	NA	96.2	97.1	92.6	93.2	103.3	0.038	99.8	102.8

[1] ND =not detectable.
[2] PES = residues remaining after exhaustive extractions.
[3] Accountability from ppm = (Total extractable + Total unextractable)/(TRRs from combustion analysis; see TABLE C.2.1) * 100. Accountability (%) = % extractable + % unextractable.
[4] NR = not reported.
[5] Not available since value of PES after enzyme extraction is not known.

C.3. Proposed Metabolic Profile

A proposed metabolic pathway for CGA-185072 in poultry is found in Appendix 1.

D. CONCLUSION

[Quinolinyl-3-^{14}C]CGA-185072 (~95 ppm or 7.419 mg/kg bw/day) was given orally to five white leghorn laying hens for 8 consecutive days and sacrificed six hours after the final dose. The majority of the dose (66%) was recovered in the excreta. Small amounts of the dose were recovered in tissues and eggs: 0.02% of the dose was recovered in the muscle and peritoneal fat, 0.04% was recovered in the liver, 0.01% was recovered in the egg white, and <0.01% was recovered in the egg yolk. Total radioactive residues, expressed as cloquintocet-mexyl equivalents, were 0.010 ppm in muscle, 0.146 ppm in liver, 0.070 ppm in fat, 0.024-0.042 ppm in egg whites, and 0.002-0.018 ppm in egg yolks.

A large majority of the radiolabel in muscle, fat, liver, and eggs was associated with the ester hydrolysis product CGA-153433 (5-chloro-8-quinolinoxyacetic acid). This metabolite comprised 50.5% TRR in the muscle, 73.3% TRR in the fat, 64.9% TRR in the liver, 78.0% TRR

in the egg whites, and 50.5% TRR in the egg yolks. One other metabolic product was identified in the liver, that being the hydroxylated product of CGA-153433 (10.2% TRR).

A metabolic scheme is provided in Appendix 1. CGA-185072 is hydrolyzed to the acid metabolite CGA-153433, which is the major residue. A small amount of further hydroxylation of the ring occurs to form an hydroxy-CGA-153433.

E. REFERENCES

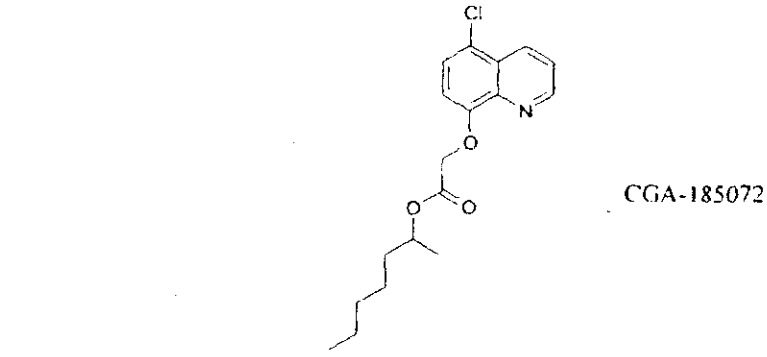
None

F. DOCUMENT TRACKING

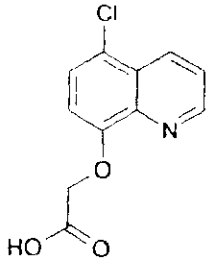
RDI: N. Dodd (11/16/05); William Wassell (11/16/05)
Petition Number: 7E04920
DP Barcode: 308470
PC Code: 700099

APPENDIX 1

PROPOSED METABOLIC PATHWAY



CGA-185072



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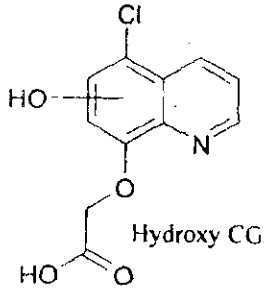
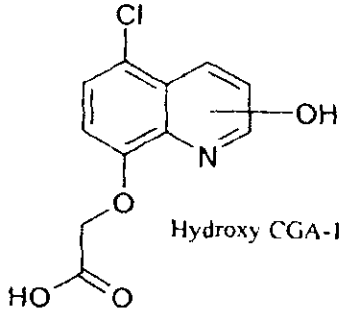


Hydroxy CGA-153433

OR



Hydroxy CGA-153433

13544

R118339

Chemical: Acetic acid, {(5-chloro-8-quinolinyl)oxy}-, 1-methylhexyl ester

PC Code:
700099
HED File Code: 11500 Petition Files Chemistry
Memo Date: 11/16/2005
File ID:
Accession #: 412-06-0009

HED Records Reference Center
2/21/2006