PP# 7E3489
4-3-89

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

APR 3 1989

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: PP#7E3489 - CGA-154281
Exemption from the Requirement of a Tolerance.
Evaluation of October 25, 1988, Amendment.
(MRID Nos. 408698-00 through -09) [DEB No. 4660]
(HED Project No. 9-0379)

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THRU: Richard D. Schmitt, Ph.D., Acting Chief
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Richard D. Schmitt

BACKGROUND

The Agricultural Division of the Ciba-Geigy Corporation has submitted an amendment consisting of a cover letter and a supplementary Section D (plant, ruminant, and poultry metabolism studies; ruminant and poultry feeding studies; additional crop field trial data from exaggerated rate applications; and a processing studies discussion). These data were submitted in response to several deficiencies as outlined and summarized in our September 28, 1988, review by F.D. Griffith, Jr. (which see). These deficiencies are repeated and listed in the body of this review in the order they appeared in the September 28, 1988, review followed by the petitioner's response, then DEB comments. Our summary of deficiencies remaining to be resolved, conclusions, and recommendations follow.

METOLACHLOR 108801

SUMMARY OF CHEMISTRY DEFICIENCIES REMAINING TO BE RESOLVED

Deficiencies in these areas need to be resolved:

- o Revised Section F, and
- o Analytical Method needs to pass a petition method validation (PMV).

CONCLUSIONS

NATURE OF THE RESIDUE - PLANTS

From studies on corn and potatoes DEB concludes that the nature of the residue in plants is adequately understood. The residue of concern is the parent compound CGA-154281. The petitioner has presented the requested studies. The deficiency has been resolved.

NATURE OF THE RESIDUE - LIVESTOCK

Based on a caprine metabolism study, DEB concludes that the nature of the residue in ruminants is adequately understood. The residue of concern is the parent compound CGA-154281. The petitioner has presented the requested study. This part of the deficiency is resolved.

The nature of the residue in poultry is adequately understood. The residue of concern is CGA-154281.The petitioner has presented the requested study. This part of the deficiency is resolved.

RESIDUE ANALYTICAL METHOD

DEB reiterates the only remaining analytical method deficiency is for the analytical method to complete a successful PMV. The deficiency continues unresolved and remains outstanding.

MAGNITUDE OF THE RESIDUE - CROP FIELD TRIALS

Residues of CGA-154281 are not expected to exceed a suggested 0.02 ppm tolerance when CGA-154281 is mixed and used with metolachlor. Crop field trials all showed no residues of CGA-154281 above the suggested 0.02 ppm tolerance or above 0.01 ppm. The petitioner has presented the requested additional crop field trial data. The deficiency is resolved.

The petitioner now needs to formalize a "tolerance proposal" in a Section F for CGA-154281 at 0.01 ppm in those crops in which metolachlor has a registered use and established tolerance. The tolerance should be for 40 CFR 180.xxx and should read "when used as a safener in formulations of the herbicide metolachlor."

MAGNITUDE OF THE RESIDUE - MEAT/MILK/POULTRY/EGGS

The petitioner has conducted a 28-day ruminant feeding study for CGA-154281. The use is characterized as one where there is no reasonable expectation of finite secondary residues; thus, meat and milk tolerances are not required. The petitioner has presented the requested ruminant feeding study. This part of the deficiency is resolved.

The petitioner has conducted a 28-day poultry feeding study for CGA-154281. This use is characterized as one where there is no reasonable expectation of finite secondary residues in poultry and eggs, i.e., 40 CFR 180.6(a)(3). The petitioner has presented the requested poultry feeding study. This part of the deficiency is resolved.

RECOMMENDATION

DEB cannot, at this time, recommend for the exemption from the requirement of a tolerance for the reasons cited above in the executive summary.

For further consideration, assuming the petition method validation is successful, the petitioner should submit a Section F for 40 CFR 180.xxx (Subpart D) proposing a tolerance of 0.01 ppm for residues of CGA-154281 when used as a safener in formulations of herbicides containing metolachlor.

DETAILED CONSIDERATIONS

NATURE OF THE RESIDUE - PLANTS

Deficiency

The nature of the residue in corn, sorghum, peanuts, soybeans, and potatoes is not adequately understood. The petitioner should conduct a radiotracer metabolism study(ies) with one or more of the target crops.

Petitioner's Response (See MRID No. 408698-02)

The petitioner responded to our plant metabolism concerns in a study titled "Metabolism of CGA-154281 in Corn and Potatoes" by F.M. Capps dated October 1988 and coded ABR-88146.

DEB Comments

The petitioner conducted a greenhouse study to maximize residues. Twenty-four separate 14-quart aluminum-lined plastic pails containing Georgia loamy sand were used for planting Superior potatoes and the same number of plastic pails were used for Funk G-4444 corn. Eight pails of each group served as controls.

^{14}C-CGA-154281, labeled in the phenyl ring with a specific activity of 40.8 uCi/mg (97.7% radiochemical pure) was mixed into soil so that 1 inch of soil top-dressed on each pail contained 0.125 lb ai/acre for a low dose rate. The ^{14}C-treated soil was mixed with nonlabeled Dual® to have 3.75 lbs ai metolachlor/acre for the 1X application rate. For the exaggerated rates of safener the petitioner used a 1.25 lbs ai/acre (10X) application rate. This treated soil was blended with Dual to give a 2.0 lbs ai metolachlor/acre application. As with the low rate, 1 inch of treated soil at the high rate was applied to eight pails each of potatoes and corn.

The petitioner also conducted a separate study injecting at the first internode 5 mg ^{14}C-phenyl-CGA-154281 into each of four corn plants. Three Green Mountain White Potato plants 7 weeks old received an injection in the first internode of 10 mg of ^{14}C-phenyl-CGA-154281.

The petitioner also provided plant metabolism data from treatment of corn cell culture with ^{14}C-CGA-154281 for 6, 24, and 72 hours.

Mature corn plants were harvested at 14, 30, 47, 60, and 81 days after treatment. Corn at the 88-day preharvest interval (PHI) at the silage stage was harvested and samples of stalk, grain, and cobs were retained. Also, mature corn at 112 days PHI was harvested and samples of the stalk, grain, and cob were retained. Immature potato plants were harvested on the same PHI schedule of 14, 30, 42, 60, and 81 days. Mature potato plants at 95 days PHI were harvested and samples of foliage and tubers were retained.

As the samples were taken they were immediately frozen, shipped on dry ice, and were retained frozen until analysis. For total ^{14}C-CGA-154281 plant samples were homogenized in a Willey Mill.

Approximately 0.2 gram were combined in a Harvey Oxidizer. The $^{14}CO_2$ was trapped in Oxosol scintillation cocktail and counted on a Beckman LSC Model 3801 for 2 minutes two times. Combustion efficiencies were based on ^{14}C-mannitol.

Five to 1200 grams (for id of TRR) whole corn plants, cobs, grain, or stalks and whole potato plants, tubers, or foliage were extracted 2X in 10 mL/gram of ACN/H_2O (9/1). The ACN was evaporated and the aqueous phase was extracted 3X with an equal volume of $CHCl_3$. The $CHCl_3$ phases were combined and analyzed as the organosoluble fraction. The aqueous fraction was hydrolyzed with cellulose 0.64 mL was mixed with equal volume of sodium acetate at pH 4.6 buffer and 500 mg to 1 gram of dry cellulose was added at 0 time and 4 hours later another 500 mg was added, then incubated overnight or at least 12 hours at 37 °C. The sample was partitioned 3 x 400 mL $CHCl_3$. The $CHCl_3$ extracts were combined and concentrated to 5 mL by rotary evaporator plus N-Evap and retained for TLC as the aqueous organosoluble extract.

The cell cultures up to 800 grams were filtered, then extracted 1 gram/10 mL CH_3OH in an Omni Mixer. The sample was filtered and the filter cake was extracted again in CH_3OH.

Further work was done on the aqueous unextractable residue. These samples were acid hydrolyzed in 10 mL/gram 2N HCl; refluxed for 2 to 24 hours, cooled, filtered, and partitioned 3X in equal volumes of $CHCl_3$. After the organic extraction, the aqueous extract was neutralized to pH 7.5 with 6N NaOH and partitioned 3X equal volume or 100 mL $CHCl_3$. The $CHCl_3$ extracts were reduced on a rotary evaporator to 0.5 mL for cells or to 10 mL for plant tissue for thin layer chromatography (TLC).

TLC determination was on Merck silica gel plates 60F-254. A number of extracts were developed in two dimensions using as SS1 $CHCl_3$/glacial acetic acid (95/5) and as SS2 $CHCl_3/CH_3OH$/formic acid/water (70/25/4/2). The compounds as radioactive spots were detected by X-ray film (Kodak X-O mat AR) or on a Berta Beta Rays Thin Layer Analyzer.

The MS identification was performed on a Finnigan Model 5100 operated in the EI mode and having a splitless capillary injection into a 30 meter J&W DB-5 0.25 μm film thickness fused silica column. From the GC the sample was introduced into the MS by direct inlet.

Total ^{14}C values from both the 1X and exaggerated rate applications in immature corn showed a rapid uptake to day 14 but a steady decline through day 60. Residues at day 14 PHI

1X application were 0.135 ppm and declined at day 60 to 0.037 ppm. From the exaggerated dose residues declined on day 14 PHI from 0.886 ppm to 0.098 ppm 60-day PHI. AS the corn plants matured and lost moisture the ^{14}C-CGA-154281 increased somewhat. At the silage stage (88-day PHI) total ^{14}C-CGA-154281 equivalents in the stalk were 0.052 ppm for the 1X dose and 0.166 ppm from the exaggerated dose. As the corn matured and dried out, the fodder stalk residues rose to 0.08 ppm from the low dose and to 0.3 ppm from the exaggerated dose. Mature corn grain values of ^{14}C-CGA-154281 equivalents from the 1X dose were 0.188 ppm and were 0.115 ppm in the exaggerated dose. The petitioner suggests this difference is due to the high phytotoxicity of Dual® at the 3.75 lb ai/A application rate. This explanation is acceptable to DEB.

A similar residue pattern for ^{14}C-CGA-154281 was noted in potatoes. Immature potato plants also showed a rapid uptake, then a steady decline from 14 days to 42 days PHI with a slight increase in residues as the plants matured and dried out. From the 1X application, residues declined from 0.131 ppm to 0.039 ppm at 42 days, rising slightly to 0.70 ppm at 81 days PHI. The mature potato plant foliage was 0.108 ppm ^{14}C-CGA-154281 equivalents and its tubers had residues of 0.032 ppm. The exaggerated 10X dose gives residues at day 14 in immature potato foliage at 0.854 ppm declining to 0.158 ppm at day 42, then rising at maturity to 0.495 ppm in foliage and 0.109 ppm in the potato tubers.

Characterization of residues via two-dimensional TLC showed a similar profile whether it was corn cell cultures, potato or corn plants, or injected plants. The various autoradiographs showed up to 20 discrete spots or metabolites of ^{14}C-CGA-154281 in corn and potatoes. There was parent CGA-154281 detected in the $CHCl_3$ organosoluble fraction of all cultures at 5 percent or 0.03 ppm. Cellulose hydrolysis did not release additional ppm of the parent ^{14}C-CGA-154281. The dominant metabolite is identified as the alcohol (3,4-dihydro-4-(hydroxyacetyl)-3-methyl-2H-1,4-benzoxazine) which accounted for 8 percent or 0.024 ppm of the residue in mature corn stalks or 47.6 percent or 2.3 ppm in the corn cell culture. The petitioner calls this metabolite "C." All other metabolites were less than this amount.

Other minor metabolites identified are metabolite "D" at 2.5 percent of the residue or 0.008 ppm. This is the benzoxazine metabolite "K." The cyclic amide metabolite is 1.0 percent of the residue or 0.003 ppm. Metabolite "L," the methylated alcohol metabolite, is also about 1 percent or 0.003 ppm.

At this point DEB concludes that the petitioner has adequately identified the residues in both corn and potatoes.

The petitioner has done organic extraction, enzyme hydrolysis, and acid hydrolysis to "free" the residues for identification. Based on cell culture analysis and analysis of corn stalks injected, the amount of unidentified/nonextractable residue at the low levels does not justify further identification. ^{14}C-CGA-154281 is rapidly taken up by both plants with the major metabolic pathway being reductive dehalogenation (glutathione dependent) to the acetylated alcohol. The acetylated alcohol is further metabolized to the cyclic amide, the methylated alcohol, and the benzoxazine metabolites. CGA-15428 can metabolize directly to the benzoxazine. The nature of the residue of CGA-154281 in plants is adequately understood. The residue of concern is the parent compound.

NATURE OF THE RESIDUE - LIVESTOCK

Deficiency

The petitioner should conduct ruminant (caprine) and poultry metabolism studies following the proposed protocols. In the final report, the petitioner should address our concerns noted below in the body of this review. DEB reiterates that at this time, the nature of the residue for CGA-154281 in livestock continues to be not fully understood.

Petitioner's Response (See MRID Nos. 408698-01, -04, and -05)

The petitioner provided the results of a ruminant metabolism study in a report titled "CGA-154281 - Nature of the Residue in Goats - Metabolism" by Diana Wu dated October 1988, and coded XBL Report No. RPT 0005.

The petitioner provided the results of a poultry metabolism study in a report titled "CGA-154281 - Nature of the Residue in Poultry - Metabolism," by F. Capps dated October 1988, and coded ABR-88142.

The petitioner presented an overall summary of all responses to EPA concerns in a study titled "CGA-154281: Summary of Research Results to Answer Deficiencies Identified in EPA's 9/28/88 Review of PP#7E3489," by F. Capps dated October 1988, and coded ABR-88153.

DEB Comments

In summary, two 2 1/2-year-old lactating goats, one an Alpine and the other a La Mancha, well into their lactation period, were received at the Agrisearch Farm Facility in Mt. Airy, Maryland on June 1, 1988. The goats were acclimated for 7 days in the test facility prior to the start of the metabolism test, with the last 2 days being in metabolism

cages 60" x 30" x 50" depth, with the cage bottom being wire screen and stainless steel. This is a specially designed cage to collect urine and feces separately. The goats were housed in a closed barn designed for year-round goat housing. The temperature was 68 to 78 °F (X = 70) and the RH ranged from 50 to 98 percent. The Alpine goat was color coded red and her log number was 8-204. The La Mancha goat was color coded blue and her log number was 8-203. The metabolism tests started on June 10 and went for 3 days with sacrifice of both goats 4 hours after last a.m. dose. The goats were fed Southern States Sweet Goat Feed "B," a commercial feed that was 16 percent protein, 2.5 percent fat, and less than 8 percent fiber. The goats were fed 0.75 kg/day of commercial feed (equal portions) and 0.75 kg of alfalfa hay. Both goats consumed the entire 1.5 kg/day ration. Water was given ad libitum. Neither the commercial feed nor the hay were analyzed for potentially interfering heavy metals, other pesticides, industrial chemicals, or alfatoxins. The Alpine goat weighed 38 kg and the La Mancha goat weighed 42 kg at the start of the metabolism test.

The goats were dosed with ^{14}C-phenyl-CGA-154281 at a level of 76.7 mg (specific activity of 21.7 uCi/mg). The petitioner filled #13 gel caps about 1/4 full of the Southern State feed, added the ^{14}C-CGA-154281, allowed the solvent to evaporate, filled the cap with feed, then sealed all seven capsules. Six caps were used in feeding and the seventh capsule was analyzed at the end of the study for a storage stability evaluation. Both goats were dosed daily just after the a.m. sample collection with a balling gun. 76.7 mg CGA-154281 in 1.5 kg of feed translates to a level of 51 ppm.

The milk samples were collected twice daily, volume recorded, then subsamples were frozen. Urine was collected in chilled containers, then frozen until analysis. Feces were collected in a similar manner. Blood samples were drawn in a.m. in a 5-mL EDTA vacutainer, then frozen until analysis. Both goats were sacrificed 4 hours after the last dose. Organs removed for analyses were liver and kidney plus at least 200 grams of back fat, omental fat, tenderloin, and leg muscle. While analysis of blood helps determine distribution of ^{14}C-CGA-154281, these results are not germane to DEB's conclusion on caprine metabolism.

4 mL of urine, 2 mL milk or blood were analyzed for total ^{14}C by direct LSC in scintillation cocktail. Tissue samples (0.2 gram) were oxidized in a Harvey Biological Materials Oxidizer with the $^{14}CO_2$ trapped in Oxosol. Samples were counted on a Beckman LSC Model 3801 using two-channel counting for 10 minutes (or 1 percent error in higher dpm). Results were reported directly in dpm (not cpm) using an external standard of ^{14}C-toluene after quench curve calculation. The samples were counted two times.

The La Mancha goat produced almost twice the amount of milk as the Alpine (2 to 4 L vs. 1.0 to 1.2 L). The effect on the dpm was that the Alpine goat's milk had a significantly higher dpm, but not 2X as high (0.059 to 0.088 ppm vs. 0.095 to 0.107 ppm of ^{14}C-CGA-154281 equivalents). No plateau of residues was reached in milk.

A majority of the radiolabeled residue was excreted in the urine and feces. The urine of the La Mancha had 30.1 ppm CGA-154281 and the feces had 26.7 ppm. The Alpine goat's urine was 27.05 ppm CGA-154281 equivalents and there was 22.76 ppm CGA-154281 in the feces. Maximum CGA-154281 blood levels were 0.019 ppm. Significant levels of residues were detected in kidney (0.71 and 0.996 ppm) and liver (0.54 and 0.49 ppm). Fat either omental or back muscles had lower amounts ranging from 0.029 to 0.042 ppm. In milk, 4 hours after last dose, ^{14}C-CGA-154281 total equivalents were 0.086 and 0.111 ppm.

The petitioner characterized and identified residues in urine, feces, liver, kidney, and milk. DEB would agree the levels of ^{14}C-CGA-154281 in fat and muscle were too low for characterization and identification. Since urine and feces are not germane to DEB's understanding of ruminant metabolism we will only briefly review CGA-154281 results in these excretory products.

100 mL of goat's milk was extracted with 300 mL of ACN. The milk solids were separated out, then extracted 2 x 100 mL ACN/H_2O (5/1), followed by another 2 x 100 mL hexane to remove the free or nonconjugated fraction. All milk ACN extracts were combined, rotary evaporated to remove ACN, then partitioned 3 x 100 mL EtoAc. After the EtoAc was removed from the aqueous phase, the aqueous portion was heated to 37 °C and incubated with 100 mL beta-glucuronidase (pH 5.2) for at least 24 hours to free up the aglycone fraction. At this point there were five fractions as follows: hexane, EtoAC-I and EtoAC-II (following beta-glucuronidase incubation), aqueous, and the milk solids left after solvent extraction.

Urine samples were handled in a similar manner. 20 mL of urine was extracted with 3 x 20 mL $CHCl_3$. Then the aqueous portion remaining was incubated for at least 24 hours at 37 °C with 100 mg beta-glucuronidase (50 mg at the start and 50 mg of beta-glucuronidase after 5 hours). The aqueous extract was partitioned 3 x 20 mL EtOAc for removal of the glycone with the aqueous fraction retained for the nonextractable fractions.

22 to 27 grams of liver or kidney were extracted with 200 mL of $CH_3OH/H_2O/CHCl_3$ (11/5/5), then centrifuged. The solids were extracted with 100 mL $CHCl_3$, then centrifuged

again. Both solvents were combined, shaken, then allowed to separate. The $CHCl_3$ became fractions I and II for the free, nonconjugated fraction after the $CHCl_3$ was solvent-exchanged into hexane and ACN. The CH_3OH/H_2O was incubated with 100 mg beta-glucuronidase (pH 5.2) at 37 °C for 24 hours, then extracted with EtoAc to recover the aglycone fraction (fraction III). The aqueous fraction was retained for the nonextractable fraction and the solid kidney or liver matrix was also retained as fraction V.

The fractions containing ^{14}C-CGA-154281 equivalent were chromatogrammed in two TLC system. The plates were Baker silica gel GF (250 u, 20 cm x 20 cm) using first $CHCl_3$/HOAc (glacial) 95/5 in one direction, then $CHCl_3/CH_3OH$/Formic Acid/ H_2O (70/25/4/2) developing system for the other direction. The plates were developed to the 15 cm line in both directions. Identification of metabolites in all caprine samples was by R_f comparison of the TLC autoradiograms from the rat study to the goat metabolism study. The developed TLC plates were scanned on an AMBIS Image Scanner for 15 minutes to 8 hours depending on the level of ^{14}C present.

In milk, the level of radioactivity in hexane reaction, i.e., free nonconjugated ^{14}C-CGA-154281, was too low for further identification (0.23% of 0.111 ppm). Fraction II, the water-soluble fraction, was found to contain the hydroxylated CGA-154281, or metabolite B_1, at 12 percent (0.012 pm) of the 0.111 ppm. This was the only metabolite identified. DEB does not consider further identification is necessary for the background scraping counts. They generally run less than 5 percent of the total count. In the aglycone fraction, small quantities of deacetylated hydroxylated metabolite A_1, the hydroxylated alcohol or metabolite A_2, the alcohol metabolite or metabolite C, and the deacetylated metabolite D were detected. Approximately 10 to 12 percent of the TRR recovered in this fraction was not identified. In the La Mancha goat this amount of unknowns was seen as eight discrete spots. DEB concludes 10 percent of 0.111 ppm divided into at least eight compounds does not warrant further identification.

In the goat livers from 45 to 53 percent of the ^{14}C residue of 0.70 ppm remained bound to the liver solids after various solvent extractions. Between 21 and 30 percent of the residue remained in the aqueous fraction after extraction with various solvents, pH adjustment, and enzymatic hydrolysis. DEB does not feel further identification work on these unknowns will help elucidate the nature of the residue. The major free metabolite identified in liver was hydroxylated phenyl ring metabolite or metabolite B_1 at 0.016 ppm. Once the enzymatic hydrolysis was completed, small amounts of metabolite B_1 (hydroxylate CGA-154281), the deacetylated

hydroxylated metabolite (A_1 metabolite), the hydroxylated alcohol (the A_2 metabolite), the deacetylated metabolite (the D metabolite), and hydroxybenzoxazine metabolite (the G metabolite). These levels ranged from 1.44 (or 0.012 ppm) to 0.22 percent (or 0.0016 ppm). Up to seven unknowns as discrete TLC spots were also seen in the EtoAc extract. Six of these spots were less than 1 percent of the TRR and the seventh spot was 1.23 percent. DEB does not feel further identification of residue is warranted for these seven spots.

The highest TRR residues were found in the goat kidneys at 0.822 ppm and 1.142 ppm. Kidney ^{14}C-CGA-154281 residue profile was more complex than liver and milk. In the ACN fraction I from the $CHCl_3$ extract there were 11 discrete metabolites (i.e., spots on the TLC plate). The $CHCl_3$ fraction was 18 percent of 0.822 ppm (0.148 ppm) with the ACN fraction being 16 percent of the 0.822 ppm (0.13 ppm). In the kidney, the parent compound was detected at 0.3 percent of 0.822 ppm. Metabolite D (deacetylated) was identified at 0.35 percent of 0.822 ppm (0.0029 ppm); metabolite B_1 (hydroxylated phenyl ring) at 0.84 percent of 0.822 ppm (0.0069 ppm), metabolite A_1 (deacetylated hydroxylated), at 0.57 percent of 0.822 ppm (0.006 ppm). From the CH_3OH/water layer after the $CHCl_3$ extraction and following the beta-glucuronidase treatment the aglycone fraction III contained 28 percent of the 0.822 ppm residue (0.23 ppm). In the aglycone fraction metabolite C is 1.28% of 0.822 ppm (0.011 ppm), metabolite B_1 is 3.02 percent of 0.822 ppm (0.025 ppm), metabolite A_1 is 5.5 percent of 0.822 ppm (0.013 ppm), and metabolite G is 2.43 percent of 0.822 ppm (0.020 ppm).

42.6 percent of the TRR remained in the aqueous extract and 10.7 percent remained unextractable in the kidney solids. DEB concludes further identification is not warranted when we consider the fractionation of the urine yields the same metabolites and also shows intact parent ^{14}C-CGA-154281. Analyses of the feces shows at least 50 percent of the parent is excreted intact. No metabolites were identified in the feces.

The nature of the residues in CGA-154281 in ruminants is adequately understood. The residue of concern is the parent compound, CGA-154281. The metabolic pathway involves hydroxylation of the aromatic ring (metabolite B_1) followed by deacetylation of the nitrogen ring. Another pathway is deacetylation of the nitrogen (metabolite D) followed by hydroxylation of the aromatic ring. Both paths lead to a deacetylated hydroxylated aromatic ring metabolite (metabolite A_1). Reductive chlorination followed by glutathione conjugation occurs on the dechlorinated product. Alcohol (metabolite C) can also have hydroxylation of the aromatic ring (metabolite A_2).

In the poultry ^{14}C-CGA-154281 metabolism study, the petitioner used White Leghorn hens 33 weeks old. These hens were purchased from Anthony Farms in Lakeland, Florida, in June 1988, and were acclimated for 8 days from June 28 to July 5, 1988 at Ciba-Geigy's test facility in Vero Beach, Florida. Each pullet was assigned an individual number, color code band and cage, and placed in individual 17" x 18" x 18" metabolism cages. The cages were designed so that excreta were collected on aluminum foil. The cages were in a controlled environment that had 24-hour continuous lighting, temperature ranging from 24 to 27 °C, and relative humidity varying from 40 to 64 percent. Dosing started on July 5, 1988, and concluded on July 12, with sacrifice 18 hours after last dose (July 13). The pullets were fed Purina Layena® crumble feed. This is a 10 percent protein chicken feed. The hens were given water ad libitum. The amount of feed consumed each day per pullet was measured and recorded. The feed intake averaged from 111 to 137 gram/day/pullet. The weight of each pullet at the start of dosing and just before sacrifice was measured and recorded. The hens initially weighed about 1.6 kg and at the conclusion weighed slightly less. DEB does not consider the weight reduction significant.

The hens were dosed with ^{14}C-CGA-154281 labeled in the phenyl ring to give a dose of 0.262 mCi/day (specific activity of 43.6 uCi/mg) with a radiolabeled purity of 97.8 percent. Each hen in the treatment phase received one capsule each at 9 a.m. each day containing 6.0 mg ^{14}C-CGA-154281 on ground corn cobs. The ^{14}C-CGA-154281 was added to the corn cobs in an acetone solution, then allowed to evaporate before the capsule was sealed. 6.0 mg of ^{14}C-CGA-154281 in about 126 g of feed translates into a 47 ppm feeding level.

Samples of excreta were collected each day in aluminum foil and stored frozen. Eggs also were collected each day with the whites and yolks being separated and shells discarded. Two of the 10 test hens had their egg samples stored individually for mass balance determination. The other eight hens had their daily egg samples composited. The egg samples from the four control hens were kept separate and were composited daily. The hens were sacrificed and the liver, kidney, heart, blood, both dark and light muscle tissue, skin plus attached fat, and the peritoneal fat pad were removed for residue analysis. While results from analysis of heart and blood help determine distribution of CGA-154281, the results are not germane to DEB's conclusions on poultry metabolism.

0.2 gram of tissues, egg white, egg yolks, and excreta were combusted in a Harvey Oxidizer with the $^{14}CO_2$ trapped in an Oxosorb scintillation cocktail. All samples were analyzed

in duplicate on a Beckman Model 3801 Liquid Scintillation Counter for 5 minutes. Combustion efficiency was determined using ^{14}C-mannitol and counting efficiency was by using an external standard from Beckman Instruments.

Low levels of ^{14}C-CGA-154281 equivalents were detected in skin with attached fat, lean muscle tissue (light and dark), and peritoneal fat. The levels ranged from 0.02 ppm (peritoneal fat) to 0.078 ppm (skin and attached fat). However, liver had between 1.62 and 1.76 ppm ^{14}C-CGA-154281 equivalents and kidney levels ranged from 0.82 to 1.33 ppm. Levels of ^{14}C-CGA-154281 in egg white were fairly consistent, showing essentially no increase or decrease over the 8 days ranging from 0.01 ppm (day 1) to 0.03 ppm (day 5) ^{14}C-CGA-154281 in egg yolks, on the other hand, did show an increase each day. At day 1, levels of ^{14}C-CGA-154281 equivalents were nondetected/0.001 ppm and rose to 0.411/0.432 ppm on days 7 and 8. Thus, it follows that ^{14}C-CGA-154281 equivalents in whole egg analyses showed the same trend as egg yolks, only the ^{14}C-CGA-154281 equivalent maximum becomes 0.146 ppm.

The petitioner separated then characterized the ^{14}C-CGA-154281 residues in tissues and eggs. 20 to 59 grams per sample of tissue were extracted 5 minutes in a Polytron using 100 mL of CH_3OH/H_2O (8:2) 3X. Between each extraction the samples were centrifuged 10 minutes at 7000 rpm in a Sovall centrifuge. The final filter cake was saved for enzymatic extraction using protease hydrolysis before extraction with CH_3OH/H_2O.

The methanol extract was partitioned with 100 mL of warm (50 °C) hexane 3X. This was the free, unbound fraction. Next, the CH_3OH was rotoevaporated off and the aqueous phase was partitioned with 100 mL of ethyl acetate 3X at pH 3. The aqueous fraction was concentrated, then transferred to an Extrelut column. After the sample equilibrated 30 minutes on the column, the column was eluted with the following solvents at 100 mL each in the following order: hexane, ethyl acetate/hexane 15/85 and 50/50; CH_3OH/ethyl acetate, 10/90, 20/80, 50/50, 80/20; CH_3OH and finally 0.125 percent TFA (aqueous)/CH_3OH 20/80.

The solid nonextractable filter cake is dissolved in 30 mL tris/HCl acid buffer, then blended for 5 minutes on a Polytron. 1 mL of protease solution (50 mg) was added to the solution three times, 4 hours apart. The sample incubated for 18 hours at 37 °C. The unbound fraction was extracted with hexane, evaporated off CH_3OH, then the aqueous remaining was partitioned with ethyl acetate.

TLC involved using silica gel plates, Merck 60F-254, 0.25 mm. The plates were developed in two dimensions. The

TLC tanks were lined with blotter paper, then saturated prior to development. The solvents were $CHCl_3$/glacial acetic acid (95/5) and $CHCl_3/CH_3OH$/formic acid/H_2O (70/ 25/4/2). Detection of the ^{14}C-CGA-154281 and its metabolites was by X-ray film (Kodak XAR-2) or a spark chamber. Quantitation by Raytest Evascanner or scrape the zone into a scintillation vial mixing with 5 mL H_2O and 10 mL of ScintiVerse cocktail and counting on the Beckmann LSC.

Various fractions from the column were chromatogrammed on a Perkin Elmer HPLC Model 410 equipped with a Waters UV detector model 440 set at 254 mm and a Beckman radioactivity detector solid flow cell, Model 171. The RAM and UV detectors were connected so that their outputs would be recorded synchronously on a 2-channel recorder. HPLC columns used were Hilbar Lichrosorb RP 18 (10 u or 5 u) 4.6 mm (i.d.) x 25 cm; Alltech ODS Spherisorb, 5 u, 4.6 mm (i.d.) x 25 cm or a Beckman Ultrasphere ODS, 5u, 10 mm (i.d.) x 25 cm with a 2.0 ml/ min flow rate on the RP 18 column Beckman column, and 1.5 mL/minute flow rate of the ODS column. Various gradient profiles were used.

The MS determination was HPLC/MS using a Vestec Model 201 and a Waters pump model 600MS. In this instance, the HPLC solvent system was isocratic 10 percent CH_3OH in 0.125 percent TFA.

The petitioner did not further identify the ^{14}C-radioactivity in lean muscle tissue (dark or light), skin with fat, peritoneal fat, and egg whites. DEB agrees further identification of these residues is not necessary. At levels of 0.08 ppm or less TRR, there is not sufficient material present to adequately characterize. DEB notes from other parts of the ^{14}C-CGA-154281 metabolism studies that are at least eight spots or metabolites present in each extract; thus, we could easily be trying to characterize a 0.01 ppm residue.

In egg white, 11 to 18.5 percent of 0.05 ppm was in the organic extract; 54 to 57 percent of 0.05 ppm (0.027 ppm) was in the aqueous extract, and 41 to 45 percent of 0.05 ppm (0.02 ppm) was not extractable. In egg yolk a different profile emerges. The composite egg yolk sample shows 18.4 percent in the organic phase, 41 percent in the aqueous phase, and 39 percent is nonextractable. The large percentage of unextractable indicates we have very polar compounds as several spots remained at or very close to the origin. The parent compound was about 10 percent of the residue at 0.027 ppm. In the excreta the same metabolites as were found in egg yolk were identified based on cochromatography of authentic metabolite standards identified in the rat metabolism study. Metabolite A_1 (the deacetylated hydroxylated ring) was 0.6 percent of 0.27 ppm or 0.014 ppm and the

A_2 metabolite (the hydroxylated ring alcohol) was 2.6 percent of 0.27 ppm or 0.002 ppm. The B_1 metabolite (the hydroxylated aromatic ring deacetylated metabolite) was 0.9 percent of 0.0035 ppm). The dechlorinated or alcohol metabolite (metabolite C) was 1.4 percent or 0.004 ppm. Metabolite E (another hydroxylated ring metabolite) was 0.7 percent of 0.27 ppm (or 0.002 ppm).

In kidney only 1.6 percent of the 1.34 ppm (0.014 ppm) was in the organic phase, 41 percent or 0.53 ppm was in the aqueous phase, and 46.7 percent or 0.65 ppm was in the nonextractable portion. 1.8 percent of the 1.76 ppm or 0.032 ppm in liver was the organic free, unbound fraction, 63.5 percent or 1.109 ppm was in the aqueous, and 31 percent of 1.76 ppm or 0.546 ppm of the ^{14}C-CGA-154781 in liver was not extractable. The same metabolites as in eggs were identified in kidney and liver. The parent compound was 0.75 percent of the residue in kidney at 0.133 ppm and 0.1 percent of the residue in liver at 0.018 ppm. Metabolite C the alcohol or dechlorinated metabolite is 0.16 percent of 1.34 ppm or 0.002 ppm of the kidney and 0.01 percent of 1.76 ppm or 0.00018 ppm of the liver. Metabolite B_1, the hydroxylated aromatic ring metabolite, is 0.12 percent of 1.34 ppm or 0.002 ppm in kidney and 0.2 percent of 1.76 ppm or 0.0035 ppm in liver. The deacetylated hydroxylated metabolite A_1 is 0.3 percent of 1.34 ppm or 0.0002 ppm of the kidney but at least 10X higher in liver at 0.49 percent of 1.76 ppm or 0.007 ppm. The metabolite A_2 or hydroxylated alcohol is also 0.03 percent of 1.34 ppm or 0.0004 ppm of the kidney and is 0.9 percent of 1.76 ppm or 0.016 ppm of the residue in liver.

In the poultry metabolism study we recognize a large percentage is not identified in the aqueous extract. The petitioner has done an adequate amount of work to attempt characterization with the various solvent washes of the Eleute column. Further identification is not necessary.

The nature of the residue of CGA-154281 in poultry is adequately understood. The residue of concern is the parent compound, CGA-154281. The metabolic pathway involves hydroxylation of the aromatic ring (metabolite B_1) followed by deacetylation at the nitrogen (metabolite A_1). Reductive dechlorination occurs producing an alcohol (metabolite C). This path proceeds with hydroxylation of the aromatic ring (metabolite A_2).

The petitioner has presented adequate metabolism studies for CGA-154281 in ruminants and poultry. The deficiency on nature of the residue is resolved. No further livestock metabolism studies are required for this petition.

RESIDUE ANALYTICAL METHOD

Deficiency

The only remaining analytical method deficiency is for the method to complete a successful PMV.

DEB Comments

It is not required for the petitioner to respond. DEB initiated a PMV request to the Analytical Chemistry Branch (ACB) on March 8, 1988. The request was to validate the parent only compound in corn forage at 0.01 and 0.02 ppm. The standard is available from the EPA Pesticides and Industrial Chemicals Repository. Its order code is F-927. Until DEB has concluded the method has completed the PMV and is suitable to enforce tolerance, the deficiency continues outstanding and remains unresolved.

MAGNITUDE OF THE RESIDUE - CROP FIELD TRIALS

Deficiency

DEB reiterates that additional field trial data for CGA-154281 on corn, peanuts, potatoes, sorghum, and soybeans are needed when the plant metabolism questions are resolved.

DEB reiterates that the petitioner should be advised that processing studies may be required for each of these commodities.

The petitioner has presented crop field trial data to show that CGA-154281, per se, is not taken up into the foliage after 29 days to a level of 0.005 ppm in green beans, radishes, sorghum, peanuts, head lettuce, peas, soybeans, potatoes, and field corn.

Petitioner's Response (See MRID No. 4086989-03)

The petitioner has presented the result of additional crop field trial studies in a report titled "Residues of CGA-154281 in 60-Day Corn and Soya Forages and Immature Potato Tubers From Field Plots Treated With Metolachlor Containing an Exaggerated Rate of CGA-154281 as an 'In the Can' Safener," by R.K. Williams dated October 1988 and coded ABR-88149.

DEB Comments

In this study the petitioner has presented the crop field trial residue results on corn (two varieties), from two field trials in IL and IA for the crop year 1988 for CGA-

154281 at the 1X rate (0.125 lb ai/A) and, and at the 5X rate (0.625 lb ai/A) at planting. The safener was mixed with Dual at 1:30 ratio and 1:6 ratio. Forage samples were harvested at 60 days PHI and were analyzed by Method AG-536A. This method has been previously reviewed, found to be suitable to gather residue data, and is the method of choice for PMV (see memorandum this petition by F.D. Griffith dated September 28, 1988).

For the crop year 1988, CGA-154281 crop field trial residue data were presented for two varieties of soybean forage 60 days PHI from IL and MO. Soybeans were mixed with Dual® at 3.75 lb ai/A (a 1:30 ratio). Another plot of soybeans was treated with CGA-154281 at the 5X rate of 0.625 lb ai/A of safener to Dual® at 3.75 lb ai/A (a 1:6 ratio). Soybean forage samples were also sampled at 60 days PHI and analyzed by Method AG-536A (see above). As with corn, the soybean forage samples were promptly frozen, packed in dry ice, and shipped to Ciba-Geigy's Greensboro labs. All samples were stored frozen at -5 °C until analysis. Sample preparation procedures as described in FDA's PAM-I were followed.

CGA-154281 crop field trial data for two trials in/on potato tubers for the 1988 crop in WA and CA were represented. Plots were treated at or just after planting with CGA-154281 at the 1X rate (0.125 lb ai/A) or 5X (0.625 lb ai/A), both mixed with Dual® at 3.75 lbs ai/A application rate. Immature potato tubers were harvested at 15 days PHI, shipped to Greensboro as described above, stored at -5 °C, and analyzed by Method AG-536A.

Control samples for each test plot were treated with Dual® at the 3.75 lbs ai/A application rate. Recovery data for CGA-154281 were presented for each crop matrix. Corn samples were spiked with CGA-154281 from 0.005 to 0.02 ppm, with recoveries ranging from 74 to 93 percent ($X = 83 \pm 9\%$, $n = 4$). Recoveries of CGA-154281 from soybeans were slightly higher and recoveries from potatoes were a little lower but statistically in the same range.

The petitioner has presented copies of 12 chromatograms, 4 for each matrix which included a standard, blank, spike, and field trial residues. DEB agrees with the petitioner's proposed limit of detection of 0.005 ppm CGA-154281, *per se*, can be recovered without interference from crop coextractives. Analysis of crop blanks by AG-325A shows the alcohol metabolite does not show coelution with CGA-154281.

The petitioner has presented adequate supporting chromatographic data for these field trial results.

No CGA-154281 residues were detected in any of the treated corn, soybean or potato samples to the 0.005 ppm level. Four samples from each field trial test site were analyzed, two for the 1X rate and two for the exaggerated rate. Results from 24 samples (8 per matrix) were presented with all results being less than 0.005 ppm for CGA-154281, per se.

At this point DEB concludes the petitioner does not need to present additional crop field trial data. The petitioner has shown the ^{14}C-CGA-154281 used for field trial tests in CA and MS for the crop year 1985 did not show residues above 0.075 ppm (CGA-154281 in corn sorghum, soybeans, potatoes, or peanuts) when treated at the maximum proposed use rate of 0.125 lb ai/A. Cold field trial data were presented for CGA-154281 at the proposed use in corn fodder, grain, and silage for the crop years 1985 and 1987 in FL for green beans, radishes, sorghum, peanuts, head lettuce, peas, soybeans, potatoes, and field corn all harvested at 30 days PHI, showed no CGA-154281 above 0.005 ppm when each matrix was treated at 0.005 ppm. Residues of CGA-154281 are not expected to exceed the petitioner's suggested tolerance of 0.02 ppm when CGA-154281 is used as directed. This part of the deficiency is resolved.

In the Summary of Research Results, the petitioner concluded a crop tolerance of 0.02 ppm should be acceptable. DEB agrees a tolerance proposal is in order. However, DEB sets tolerances no higher than necessary and at levels the residue analytical method shows is reproducible. DEB suggests the petitioner propose in a Section F a tolerance, not an exemption from the requirement for a tolerance, for CGA-154281 at 0.01 ppm on all crops which have registered uses for metolachlor and established metolachlor tolerances.

Since no residues were detected in any of the raw agricultural commodities from either the proposed use rate or the exaggerated use rate processing studies are not required. Food and/or feed additive tolerance are not required. This part of the deficiency is resolved.

MAGNITUDE OF THE RESIDUE - MEAT/MILK/POULTRY/EGGS

Deficiency

The petitioner should conduct the caprine and poultry feeding studies following the proposed protocols, and in the final report, the petitioner should address our concerns noted below.

Petitioner's Response (See MRID Nos. 408698-06, -07, -08, and -09)

The petitioner provided the results of a ruminant feeding study in a report titled "^{14}C-CGA-154281 Feeding Study in Dairy Goats," by D.L. Merricks dated October 10, 1988 and coded Agrisearch Project No. 12128.

The petitioner provided results of storage stability for the doses given goats in the feeding study in a report titled "^{14}C-CGA-154281 Feeding Study in Dairy Goats - Stability in Dosing Capsules After 28 Days," by A. Merritt dated October 10, 1988.

The petitioner provided the results of a poultry feeding study in a report titled "^{14}C-CGA-154281 Feeding Study in Laying Hens," by D.L. Merricks dated September 15, 1988 and coded Agrisearch Project No. 12127.

The petitioner provided results of storage stability for doses given poultry in the feeding study in a report titled "^{14}C-CGA-154281 Feeding Study in Laying Hens - Stability in Dosing Capsules after 28 Days," by A. Merritt and dated August 26, 1988.

DEB Comments

For the ruminant feeding study the petitioner used five Nubian lactating goats that were 2 to 3 years old. The goats were received at the Agrisearch Farm Facility, Mt. Airy, Maryland, in apparent good health. Starting on August 18, 1988, the five goats were acclimated for 6 days at the research facility, including 2-day acclimation in metabolism cages measuring 60" x 30" x 50". In addition to the cage, these goats were also acclimated to the balling gun dosing technique prior to the start of the feeding study. The study was conducted in a closed barn suitable for year-round housing of goats. The temperature varied from 60 to 80 °F and the relative humidity varied from 54 to 100 percent. The goats had natural light and dark for the study. The goats were identified by I.D. on cage as there was only one goat per cage and only one goat out of a cage at a time.

The goats were feed 1 kg of alfalfa hay per day and 1 kg of Southern States Sweet Goat Feed "B," a commercial goat feed that was 16 percent protein, 2.5 percent fat, and less than 8 percent fiber. All five goats consumed the entire ration each day. Water was given ad libitum. Neither the commercial feed nor the hay were analyzed for potentially interfering heavy metals, other pesticides, industrial chemicals, or alfatoxins. At the start of the feeding study the goats weighed between 34 and 44 kg and at sacrifice four of the goats weighed the same with the fifth goat gaining 0.5 kg.

The goats were dosed with ^{14}C-phenyl-CGA-154281 at a level of 0.09 mg/day for 28 days. 107 prefilled #13 gelatin caps were used in the study with 0.9 mg of CGA-154281 in acetone. The specific activity of the ^{14}C-CGA-154281 was 43.6 uCi/mg. All goats were dosed daily after the a.m. milking. 0.09 mg of CGA-154281 in 2.0 kg of feed is 0.045 ppm level in the feed. Dosing of the #13 gel caps was by a balling gun.

No urine or feces were collected. Milk samples were collected twice daily, volume recorded, and subsamples were frozen. The first four goats were sacrificed about 20 hours following dosing on days 7, 14, 21, and 28 of the study. The final goats had a 3-day withdrawal, then sacrifice 20 hours later. At sacrifice kidneys, liver, omental and peripheral fat, tenderloin, and round muscle were removed for analysis. Prior to freezing, all samples were cut into about 1-inch cubes, then the cubes were frozen. While the petitioner also drew blood samples (EDTA), the results of CGA-1542811 blood analysis are not germane to DEB's conclusions on caprine feeding studies.

Prior to radioanalysis, the tissues were homogenized with dry ice in a Hobart food chopper. 0.2 gram of tissue sample and 2 mL of milk were combusted in a R.J. Harvey Biological Materials Oxidizer. The $^{14}CO_2$ was trapped in an Oxosol scintillation cocktail. All samples were counted and quantitated on a Beckman Liquid Scintillation Spectrophotometer Model LS 3801 using two-channel counting for 5 minutes. Results were reported directly in dpm, not cpm, using the internal standard after quench curve calculation. The samples were counted two times.

While the level of milk produced by the goats varied, it did not affect the levels of ^{14}C-CGA-154281 detected in any milk samples. The level of ^{14}C-CGA-154281 detection in milk was 0.0001 ppm. Samples counted were from the predose day, days 1, 5, 7, 14, 28, withdrawal day 1, and withdrawal day 3. No ^{14}C-CGA-154281 equivalents were detected above 0.0002 ppm. Only 2 of the 31 samples had 0.0002 ppm; the rest were all < 0.0001 ppm. The level of ^{14}C-CGA-154281 detection in goat kidney liver fat and muscle was 0.0002 ppm. None of these samples from any of the five goats showed levels of ^{14}C-CGA-154281 above the limit of detection, i.e., 0.002 ppm.

As the petitioner's intent is to add CGA-154281 to metolachlor, it is conceivable that the percent of all livestock diet containing CGA-154281 based on established metolachlor uses is at the 100 percent level. Taking into account our suggested tolerance level of 0.01 ppm in crops and a finding of 0.0002 ppm residue in milk and no detectable in meat (<0.002 ppm) from a 0.045 ppm feeding level, we conclude that there will be no problem of secondary residues

in meat and milk. These low levels of CGA-154281 in goats do not warrant regulation.

DEB concludes the petitioner has conducted a proper CGA-154281 ruminant feeding study. We characterize the proposed use under 40 CFR 180.6(a)(3) in that it is not possible to establish with certainty whether finite residues will be incurred, but there is no reasonable expectation of finite residues. Secondary tolerances of CGA-154281 are not necessary in meat and milk. This part of the deficiency is resolved.

The petitioner conducted a separate study to show that the ^{14}C-CGA-154281 in solution was mixed with corn cob, allowed to evaporate, then sealed. The ^{14}C-CGA-154281 remained as ^{14}C-CGA-154281 for the entire 28 days. For the goat study, the recovery level was 99.5 percent, and for the poultry study the recovery was 101.68 percent.

For the poultry feeding study, the petitioner used 20 White Leghorn hens that were approximately 37 weeks old. These hens were received at Ciba-Geigy's Research Farm in Vero Beach, Florida from Anthony Farms, Lakeland, Florida, in June 1988. The hens were placed in individual metabolism cages measuring 12" x 18" x 18". All hens were acclimatized to the metabolism cages for 7 days prior to the start of dosing on July 22, 1988. The hens were housed in an enclosed facility where the temperature ranged from 24 to 27 °C and the relative humidity was 50 to 65 percent. There was continuous lighting throughout the test period. Each hen received an individual identification number on a leg band. That same number was attached to the cage. The pullets were fed 160 gram/hen/day of Purina's Layena crumble feed. This is a 16 percent protein commercial feed. Water was consumed ad libitum. There are no known interfering heavy metals, other pesticides, industrial chemicals, or alfatoxins present in the feed. This was confirmed at autopsy as no gross abnormalities were observed. The hens were divided into a control group of five hens and a test group of 15 hens. The control hens at the start of the study weighed an average of 1.69 kg and until sacrifice weights ranged from 1.68 to 1.82 kg. The test hens at the start of the study weighed an average of 1.73 kg and until sacrifice the weights ranged from 1.68 to 1.74 kg. DEB does not consider these weight changes significant.

The 15 hens were dosed with ^{14}C-CGA-154281 at a level of 0.000846 mg/day for 28 days. The control hen also received a placebo cap each day containing only finely ground corn cobs.

Prefilled capsules were used in the study. The caps were partly filled with corn cobs, the acetone solution containing ^{14}C-CGA-154281 at 0.000846 mg was added, solvent

evaporated off, and capsules were sealed. The specific activity of ^{14}C-CGA-154281 was 43.6 uCi/mg. Each test hen was given either a dose cap or a placebo cap each a.m. 0.84 m/40 g of feed is 0.053 ppm level in the feed.

No excreta was collected. Egg collection was daily prior to dosing. Production records were maintained. Eggs from each group were pooled, blended as whole egg with shells discarded, and stored frozen. Egg samples from predose (0 day), days 1, 5, 7, 14, 21, 27, 28, and withdrawal days 1, 2, and 3 were analyzed. After start of dosing on days 7, 14, 21, 28, and withdrawal day 3, one control hen and three dosed hens were sacrificed. All of the liver and kidney were removed and as much breast muscle, thigh muscle, body fat, and skin with its fat pad were removed for analysis. Prior to freezing all tissue samples were cut into about 1-inch cubes, then the cubes were frozen.

Prior to radioanalysis, the tissues were homogenized with dry ice in a Hobart Food Chopper. 0.2 gram of tissue or whole egg were combusted in a R.J. Harvey Biological Materials Oxidizer. The $^{14}CO_2$ was trapped in Oxosol scintillation cocktail. All samples were counted and quantitated on a Beckman Liquid Scintillation Spectrophotometer Model LS 3801 using two-channel counting for 5 minutes. Results were reported directly in dpm, not cpm, using the internal standard after quench curve calculation. The samples were counted 2 times.

Egg production remains high during the test period for the treated hens ranging from 90 to 100 percent. The levels of ^{14}C-CGA-154281 equivalents in all whole egg samples was less than 0.002 ppm. The egg samples counted were predose or 0 day, days 1, 5, 7, 14, 21, and 28, plus withdrawal days 1, 2, and 3. The levels of ^{14}C-CGA-154281 in poultry muscle (light and dark), skin with fat, fat, and kidney were all $\leq$ 0.002 ppm. Only the kidney sample at day 28 was 0.002 ppm ^{14}C-CGA-154281 equivalents. Liver samples on the other hand all showed ^{14}C-CGA-154281 equivalents from 0.003 ppm on days 7 and 14, to 0.004 ppm on days 21 and 28. CGA-154281 was rapidly eliminated at withdrawal day 3 and the liver levels were < 0.002 ppm.

As the petitioner's intent is to add CGA-154281 to metolachlor, it is conceivable that the percent of all poultry (laying hens, broilers, turkeys) diets containing CGA-154281 based on established metolachlor uses is at the 100 percent level. Taking into account our suggested 0.01 ppm tolerance level in crops and finding of <0.002 ppm in eggs and tissues (except up to 0.04 ppm in liver) from a feeding of 0.053 ppm, we conclude that there will be no problem of secondary residues in poultry tissue and eggs.

These low levels of CGA-154281 in poultry do not warrant regulation.

DEB concludes the petitioner has conducted a proper CGA-154281 poultry feeding study. We characterize the proposed use under 40 CFR 180.6(a)(3) in that it is not possible to establish with certainty whether finite residue will be incurred, but there is no reasonable expectation of finite residues. Secondary tolerances of CGA-154281 are not necessary in poultry and eggs. This part of the deficiency is resolved.

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cc:R.F.,Circu(7),Reviewer(FDG),PP#7E3489,ISB/PMSD (Eldredge).

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