ANALYTICAL METHOD FOR THE DETERMINATION
OF CGA-152005 IN MEAT, MILK, BLOOD, AND EGGS, BY
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
INCLUDING VALIDATION DATA

DATA REQUIREMENT
EPA GUIDELINE NUMBER 171-4(d)

AUTHOR
DANIEL D. CAMPBELL

STUDY COMPLETED
MARCH 31, 1992

PERFORMING LABORATORY
Biochemistry Department
Ciba Plant Protection
Ciba-Geigy Corporation
Greensboro, NC 27419

LABORATORY PROJECT IDENTIFICATION
ANALYTICAL METHOD AG-592

VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 126

Ciba Plant Protection
Ciba-Geigy Corporation
Post Office Box 18300
Greensboro, NC 27419

TOTAL NUMBER OF PAGES IS 126
STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10 (d) (1) (A), (B) or (C).

Company: Ciba Plant Protection, Ciba-Geigy Corporation

Company Representative: Thomas J. Parshley

Title: Senior Regulatory Manager

[Signature]

4/4/94

These data are the property of Ciba-Geigy Corporation and, as such, are considered confidential for all purposes other than compliance with FIFRA Section 10.

Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality that may exist under any other statute or in any other country.

SUBMITTER/SPONSOR:
Ciba Plant Protection
Ciba-Geigy Corporation
Post Office Box 18300
Greensboro, NC 27419
STATEMENT CONCERNING GOOD LABORATORY PRACTICE

The Good Laboratory Practices Compliance Statement regarding EPAs GLP Standards (40 CFR Part 160) provided on page twenty-seven (27) of this volume and signed by the Study Director is truthful and accurate.

Dennis S. Hackett, Ph.D., Director
Biochemistry Group
Agent of Submitter/Sponsor

Date

SUBMITTER/SPONSOR:
Ciba Plant Protection
Ciba-Geigy Corporation
Post Office Box 18300
Greensboro, NC 27419
<table>
<thead>
<tr>
<th>TITLE</th>
<th>PAGE NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG-592: Analytical Method for the Determination of CGA-152005 In Meat, Milk, Blood, and Eggs by High Performance Liquid Chromatography including Validation Data</td>
<td>5</td>
</tr>
<tr>
<td>Appendix I: Protocol 26-92 and Amendment 1</td>
<td>58</td>
</tr>
<tr>
<td>Appendix II: Separate Documents Accompanying this Report</td>
<td>117</td>
</tr>
<tr>
<td>Appendix III: Residue Test Report RI-MV-001-92</td>
<td>118</td>
</tr>
</tbody>
</table>

SUBMITTER/SPONSOR:
Ciba Plant Protection
Ciba-Geigy Corporation
Post Office Box 18300
Greensboro, NC 27410

TOTAL NUMBER OF PAGES IS 126
CIBA-GEIGY CORPORATION
AGRICULTURAL DIVISION
RESIDUE CHEMISTRY DEPARTMENT
410 SWING ROAD
P. O. BOX 18300
GREENSBORO, NC 27419

ANALYTICAL METHOD AG-592

ANALYTICAL METHOD FOR THE DETERMINATION
OF CGA-152005 IN MEAT, MILK, BLOOD, AND EGGS BY
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
INCLUDING VALIDATION DATA

PROJECT NUMBER: 168982  PROTOCOL: 26-92

STUDY INITIATION DATE: February 10, 1992

SUBMITTED BY: Daniel D. Campbell

TITLE: Associate Chemist

SIGNATURE: [Signature]

APPROVED BY: R. K. Williams

TITLE: Manager, Method Development

SIGNATURE: [Signature]

DATE: 3/31/92

STUDY DIRECTOR: Robert A. Yokley, Ph.D.

SIGNATURE: [Signature]

COMPLETION DATE: 3-31-92
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>I. SUMMARY AND INTRODUCTION</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Scope</td>
<td>7</td>
</tr>
<tr>
<td>B. Principle</td>
<td>7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>II. MATERIALS AND METHODS</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Apparatus</td>
<td>8</td>
</tr>
<tr>
<td>B. Reagents</td>
<td>9</td>
</tr>
<tr>
<td>C. Analytical Procedure</td>
<td>10</td>
</tr>
<tr>
<td>1. Extraction</td>
<td>10</td>
</tr>
<tr>
<td>2. Partitioning</td>
<td>11</td>
</tr>
<tr>
<td>3. Alumina-A Sep-Pak Cleanup</td>
<td>12</td>
</tr>
<tr>
<td>D. Instrumentation</td>
<td>14</td>
</tr>
<tr>
<td>1. Description and Operating</td>
<td>14</td>
</tr>
<tr>
<td>2. Standardization</td>
<td>15</td>
</tr>
<tr>
<td>E. Interferences</td>
<td>15</td>
</tr>
<tr>
<td>F. Confirmatory Techniques</td>
<td>15</td>
</tr>
<tr>
<td>G. Time Required</td>
<td>15</td>
</tr>
<tr>
<td>H. Modifications and Potential</td>
<td>15</td>
</tr>
<tr>
<td>Problems</td>
<td></td>
</tr>
<tr>
<td>1. Emulsion Formation</td>
<td>15</td>
</tr>
<tr>
<td>2. Heat</td>
<td>16</td>
</tr>
<tr>
<td>3. Degradation in Methanol</td>
<td>16</td>
</tr>
</tbody>
</table>
TABLE OF CONTENTS
(Continued)

I. Preparation of Standard Solutions ... 16
   1. Preparation of Analytical Standards ..................... 16
   2. Preparation of Fortification Standards .................. 16

J. Methods of Calculation ...................... 17
   1. Linear Regression Analysis ...... 17
   2. Calculation of Sample Concentration .................... 17

K. Fortification Experiments ............... 18
   1. Substrate Fortification .......... 18
   2. Calculation of Procedural Recovery .................... 18

III. RESULTS AND DISCUSSION .................. 19

IV. CONCLUSION ................................ 22

V. CERTIFICATION AND CERTIFICATION OF GOOD LABORATORY PRACTICES .......... 23

VI. QAU STATEMENT ............................ 24

VII. LIST OF TABLES AND FIGURES ............. 25

TABLE I. LIQUID CHROMATOGRAPHIC OPERATING PARAMETERS FOR THE ANALYSIS OF CGA-152005 .... 25

TABLE II. TYPICAL STANDARDIZATION DATA FOR CGA-152005 (FROM THE ANALYSIS OF POULTRY LEAN MEAT) .......... 26

TABLE III. RECOVERY RESULTS FOR CONTROL AND CGA-152005 FORTIFIED CONTROL SUBSTRATES USING METHOD AG-592 ............. 27

TOTAL NUMBER OF PAGES IS 126
### TABLE IV.
**PRECISION OF ANALYTICAL METHOD AG-592 AS DEMONSTRATED BY THE RESULTS OF ANALYZING $^{14}$C-CGA-152005 CONTAINING SUBSTRATES**

<table>
<thead>
<tr>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
</tr>
</tbody>
</table>

### TABLE V.
**SUMMARY TABLE FOR THE EXTRACTABILITY, ANALYSIS, AND ACCOUNTABILITY OF $^{14}$C-CGA-152005 TREATED SUBSTRATES USING METHOD AG-592**

<table>
<thead>
<tr>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
</tr>
</tbody>
</table>

### FIGURE 1.
**STRUCTURE AND CHEMICAL NAME OF CGA-152005**

<table>
<thead>
<tr>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
</tr>
</tbody>
</table>

### FIGURE 2.
**ANALYTICAL PROCEDURE FLOWCHART FOR AG-592**

<table>
<thead>
<tr>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
</tr>
</tbody>
</table>

### FIGURE 3.
**TYPICAL STANDARD CHROMATOGRAMS (FROM POULTRY LEAN MEAT ANALYSES)**

<table>
<thead>
<tr>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
</tr>
</tbody>
</table>

### FIGURE 4.
**CALIBRATION PLOT OF THE STANDARDS OBTAINED FROM THE ANALYSIS OF POULTRY LEAN MEAT**

<table>
<thead>
<tr>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
</tr>
</tbody>
</table>

### FIGURE 5.
**CHROMATOGRAMS OF THE REAGENT BLANKS FROM THE ANALYSIS OF (A) POULTRY LIVER, (B) POULTRY PERITONEAL FAT, (C) BEEF TENDERLOIN, AND (D) BEEF BLOOD**

<table>
<thead>
<tr>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
</tr>
</tbody>
</table>

### FIGURE 6.
**REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF POULTRY LEAN MEAT FOR CGA-152005 USING METHOD AG-592**

<table>
<thead>
<tr>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
</tr>
</tbody>
</table>
TABLE OF CONTENTS
(Continued)

FIGURE 7. REPRESENTATIVE CHROMATOGRAMS
FROM THE ANALYSIS OF EGGS
FOR CGA-152005 USING METHOD
AG-592 ....................... 37

FIGURE 8. REPRESENTATIVE CHROMATOGRAMS
FROM THE ANALYSIS OF GOAT
LIVER FOR CGA-152005 USING
METHOD AG-592 ............... 38

FIGURE 9. REPRESENTATIVE CHROMATOGRAMS
FROM THE ANALYSIS OF GOAT
OMENTAL FAT FOR CGA-152005
USING METHOD AG-592 ........ 39

FIGURE 10. REPRESENTATIVE CHROMATOGRAMS
FROM THE ANALYSIS OF GOAT
ROUND FOR CGA-152005 USING
METHOD AG-592 ............... 40

FIGURE 11. REPRESENTATIVE CHROMATOGRAMS
FROM THE ANALYSIS OF GOAT
KIDNEY FOR CGA-152005 USING
METHOD AG-592 ............... 41

FIGURE 12. REPRESENTATIVE CHROMATOGRAMS
FROM THE ANALYSIS OF GOAT
MILK FOR CGA-152005 USING
METHOD AG-592 ............... 42

FIGURE 13. REPRESENTATIVE CHROMATOGRAMS
FROM THE ANALYSIS OF POULTRY
LIVER FOR CGA-152005 USING
METHOD AG-592 ............... 43

FIGURE 14. REPRESENTATIVE CHROMATOGRAMS
FROM THE ANALYSIS OF POULTRY
PERITONEAL FAT FOR CGA-152005
USING METHOD AG-592 ........ 44

TOTAL NUMBER OF PAGES IS 126
TABLE OF CONTENTS

(Continued)

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>DESCRIPTION</th>
<th>PAGE NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIGURE 15.</td>
<td>REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF LIVER FOR CGA-152005 USING METHOD AG-592</td>
<td>45</td>
</tr>
<tr>
<td>FIGURE 16.</td>
<td>REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF KIDNEY FOR CGA-152005 USING METHOD AG-592</td>
<td>46</td>
</tr>
<tr>
<td>FIGURE 17.</td>
<td>REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF TENDERLOIN FOR CGA-152005 USING METHOD AG-592</td>
<td>47</td>
</tr>
<tr>
<td>FIGURE 18.</td>
<td>REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF ROUND FOR CGA-152005 USING METHOD AG-592</td>
<td>48</td>
</tr>
<tr>
<td>FIGURE 19.</td>
<td>REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF OMENTAL FAT FOR CGA-152005 USING METHOD AG-592</td>
<td>49</td>
</tr>
<tr>
<td>FIGURE 20.</td>
<td>REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF PERITONEAL FAT FOR CGA-152005 USING METHOD AG-592</td>
<td>50</td>
</tr>
<tr>
<td>FIGURE 21.</td>
<td>REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF BLOOD FOR CGA-152005 USING METHOD AG-592</td>
<td>51</td>
</tr>
<tr>
<td>FIGURE 22.</td>
<td>REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF MILK FOR CGA-152005 USING METHOD AG-592</td>
<td>52</td>
</tr>
</tbody>
</table>

VIII. REFERENCES .............................................. 53
I. SUMMARY AND INTRODUCTION

A. SCOPE

This method is for the determination of residues of CGA-152005 (see Figure 1) in animal tissues, milk, blood, and poultry eggs. The limit of detection of this method is 0.60 ng of CGA-152005 and the limit of determination is 0.05 ppm for tissues, eggs, and blood and 0.01 ppm for milk.

B. PRINCIPLE

A 10-g subsample of substrate is homogenized with 90% acetone:10% 0.1% sodium bicarbonate aqueous solution. After filtering, the volume is reduced under vacuum until only the aqueous portion remains. The sample is diluted with 25 ml 0.4% sodium carbonate and partitioned against methyl tert-butyl ether:hexane (1:1). The aqueous phase is acidified with 20 ml 0.8% phosphoric acid, followed by the addition of 20 ml of saturated aqueous salt solution. The compound is extracted by partitioning with dichloromethane:hexane (1:1). The organic portion is dried with sodium sulfate, reduced to about 10 ml and added to a preconditioned alumina-A Sep-Pak column. After column washes with hexane, ethyl acetate, and 5% acetonitrile in ethyl acetate, the compound is eluted with methanol:acetonitrile (50:40). The eluate is concentrated to just dryness. One ml of acetonitrile is added, followed by one ml of picopure water, and the sample is filtered through a 0.45 micron Acrodisc filter. The filter is rinsed with 1 ml of acetonitrile:water (1:1). The sample is analyzed by high performance liquid chromatography (HPLC) using a narrow bore reverse phase column (Supelcosil LC-18-DB) with a mobile phase of acetonitrile:0.05% phosphoric acid in picopure water (40:60). Detection is by UV at 225 nm. A flowchart of the method is shown in Figure 2.
II. MATERIALS AND METHODS

A. APPARATUS

1.0 Analytichem Reservoir (Analytichem # 1213-1-1012 or equivalent)

2.0 Boston wide mouth round, 8-oz. (Penn Bottle Co.)

3.0 Filter, Acrodisc LC PVDF, 0.45 μm, 25.0 mm (Product #4408)

4.0 Filter Paper, Folded Reeve Angel #802,18.5 cm

5.0 Flasks, Erlenmeyer, 250 and 500-ml (Fisher # 10-047C and 10-047E)

6.0 Flasks, Round Bottom, 100-ml, 250-ml, and 1000-ml (Fisher #10-067C, 10-067E, and 10-067H)

7.0 Funnel, Fluted Pyrex (Fisher # 10-329C or equivalent)

8.0 Funnel, Separatory, 125-ml, and 250-ml, with teflon stopcock (Fisher #10-437-10B, and 10-437-10C)

9.0 Graduated cylinder, 10-ml, 25-ml, 50-ml, 100-ml, 250-ml (Fisher #08-551A, 08-551B, 08-551C, 08-551D, and 08-551E)

10.0 Homogenizer, Polytron (Brinkman Instruments or equivalent)

11.0 Pasteur pipets (Kimble 13-678-30B, 13-678-30C)

12.0 Pipets, volumetric class A

13.0 Rotary Evaporator, Buchii or equivalent, with warm water bath (Buchler Instruments or equivalent), refrigerated recirculating bath (Endocal or equivalent), and aspirator pump (Cole-Parmer or equivalent)

14.0 Sep-Pak Alumina-A Cartridges, (Waters #51800)
15.0 Syringe, Hamilton Gastight, Luer Tip, 1-ml (Fisher #14-824-24 or equivalent)

16.0 Ultrasonicator (Heatsystems Inc. or equivalent)

17.0 Vials, crimtop borosilicate (Sun Broker Inc. or equivalent)

18.0 Kontes 100 ml Rotary Evaporator Trap (Fisher catalog K570200-0124 or equivalent)

B. REAGENTS

1.0 Acetone, OPTIMA grade (Fisher # A949-4 or equivalent)

2.0 Acetonitrile, HPLC grade (Fisher # A998-4 or equivalent)

3.0 Dichloromethane, HPLC grade (Burdick & Jackson #300-4 or equivalent)

4.0 Ethyl Acetate, HPLC grade (Fisher # E195-4 or equivalent)

5.0 Hexane, HPLC grade (Fisher # H302-4 or equivalent)

6.0 Methanol, HPLC grade (Fisher # A452-4 or equivalent)

7.0 Methyl tert-butyl ether, HPLC grade (Fisher #E127-4)

8.0 Phosphoric acid, HPLC grade (Fisher #A260 or equivalent)

9.0 Sodium bicarbonate, Certified ACS grade (Fisher #S233-3 or equivalent)

10.0 Sodium carbonate, Certified ACS grade (Fisher #S263-3 or equivalent)

11.0 Sodium chloride, Certified ACS grade (Fisher #S271-3 or equivalent)

12.0 Water, HPLC grade (picopure or equivalent)

C. ANALYTICAL PROCEDURE

1.0 Extraction

1.1 Meat, Blood, Milk, and Eggs

Weigh 10 grams of tissue slices, well mixed blood or egg homogenate, or 50 grams milk into a tared 8-oz. glass bottle. Add 90 ml of 90% acetone:10% 0.1% sodium bicarbonate in picopure water. Homogenize for 30 seconds with the polytron at medium speed. Return any tissues remaining on the polytron blade back to the 8-oz. bottle. Filter through a Reeve Angel #802 filter in a longstem funnel into a 250-ml Erlenmeyer flask. NOTE: The second extraction is not required for the milk samples. Remove the filter paper after all solvent has passed through and place it back into the 8-oz. bottle. Add another 90 ml of 90% acetone:10% 0.1% sodium bicarbonate and homogenize with the polytron for 30 seconds. The generator should be at the bottom of the filter paper and will homogenize the substrate left on the paper, but not the filter itself. A polytron setting slower than the one used in the first extraction may be required. Pour the homogenate through a new Reeve Angel #802 filter and collect with the first extract. Measure and record the volume of the combined extracts for later calculations.
2.0 Partitioning

2.1 Transfer the extract from section 1.1 to a 1000-ml round bottom flask and reduce the solvent by rotary evaporation under vacuum without the use of the water/bath. A rotary evaporator trap is essential during extract evaporation. Then, after about two minutes, lower the flask into a 38 – 40°C warm water bath.

CAUTION: While bumping does not typically occur in the early stages of the extract reduction, bubbling will occur when most of the organic solvent has been removed. The addition of 10 ml of ethyl acetate to the sample will help if excessive bubbling occurs. **Remove the sample when only the aqueous portion remains.** Transfer the solution to a 125-ml separatory funnel.

2.2 Add 10 ml of 0.4% sodium carbonate to the 1000-ml round bottom flask from Section C.2.1, swirl and sonicate to dissolve remaining residues. Transfer to the same 125-ml separatory funnel containing the sample. Repeat the rinse of the 1000-ml round bottom flask with another 10 ml of 0.4% sodium carbonate, and a third rinse of 5 ml. Add 30 ml of methyl tert-butyl ether (MtBE):hexane (1:1) to the 125-ml separatory funnel and shake for one minute, taking care to vent the funnel.

Allow the two layers to separate, breaking any emulsions that may form by agitating with a glass rod or by applying a small amount of heat. Drain the lower aqueous layer and any persistent
emulsions into a 250-ml Erlenmeyer flask and discard the organic layer. Pour the aqueous phase back into the 125-ml separatory funnel and repeat the extraction with 30 ml of MtBE:hexane (1:1). Collect the aqueous layer in the same Erlenmeyer flask.

2.3 Add 20 ml of 0.8% H_{2}PO_{4} (in picopure water) and 20 ml of picopure water saturated with sodium chloride to the aqueous phase in the Erlenmeyer flask from section C.2.2. Transfer to a 250-ml separatory funnel and extract 3 times by partitioning with 60 ml dichloromethane:hexane (1:1), taking care to vent the funnel. The first 60 ml of dichloromethane:hexane (1:1) used for the partitioning should also be used to rinse the 250-ml Erlenmeyer flask. Treat emulsions that form as was done in section C.2.2, and combine and save the upper organic layers in a separate 500-ml Erlenmeyer flask.

3.0 Alumina-A Sep-Pak Cleanup

3.1 Attach a 50-ml Analytichem reservoir to an Alumina-A Sep-Pak column, and condition the column with the following rinses: 25 ml methanol: acetonitrile (60:40), 15 ml ethyl acetate, and 25 ml dichloromethane (DCM):hexane (1:1). Do not let the column dry between rinses and prior to applying the sample.

3.2 Add approximately 15 grams of sodium sulfate directly to the organic phase from section C.2.3 to bind any water in the extract, and transfer to a 500
ml round bottom flask. Rinse the sodium sulfate 3 times with small volumes (-3-5 ml) of DCM:hexane (1:1). Reduce the volume of the sample under vacuum by rotary evaporation in a 35°C warm water bath to 5 to 10 ml, taking care not to let the sample go to dryness.

3.3 Add the sample from section C.3.2 to the conditioned Alumina-A Sep-Pak column. Rinse the round bottom flask 3 times with 1 to 2 ml of DCM:hexane (1:1), pouring each rinse into the reservoir above the Alumina-A column. Allow the sample and washes to drip through the column by gravity only. The flow may occasionally stop and can be restarted by applying gentle pressure to the top of the reservoir with a pipet bulb. Wash with the following, being careful to insure that the column does not go dry between rinses. Collect the eluate in a 100-ml round bottom flask.

Wash with 15 ml hexane (discard)

Wash with 25 ml ethyl acetate (discard)

Wash with 15 ml 5% acetonitrile in ethyl acetate (discard)

Elute with 30 ml 60:40 methanol: acetonitrile (collect)

3.4 Reduce the eluate volume from section C.3.3 to just dryness under vacuum by rotary evaporation in a 35°C water bath. This step must be done soon after elution from the
Alumina-A column since preliminary reports indicate that CGA-152005 is unstable in methanol for prolonged periods of time. When the sample has just reached dryness, remove it from the rotary evaporator and add 1 ml acetonitrile. Swirl and sonicate to dissolve all residues. Add 1 ml picopure water and swirl and sonicate a second time.

3.5 Prewash a 0.45 μm, 25mm Acrodisc PVDF HPLC syringe filter with 3 X 1ml acetonitrile: picopure water (1:1). Filter the sample from section C.3.4 slowly through the Acrodisc using a 1-ml glass syringe. Add 1 ml acetonitrile: picopure water (1:1) to the round bottom flask, swirl and pass through the same filter as a rinse. Mix the sample well and transfer 1 to 2 ml to a borosilicate crimp-top vial for analysis by HPLC. Increase the final dilution volume for samples containing higher concentrations of CGA-152005 such that the peak heights obtained by LC analysis will be within the range of the calibration standards discussed in sections I.1.0 and J.1.0.

D. INSTRUMENTATION

1.0 Description and Operating Conditions

Residues of CGA-152005 are determined by HPLC on a narrow bore base deactivated Supelco C-18 column (LC-18-DB). The mobile phase is 60% 0.05% phosphoric acid in picopure water:40% acetonitrile at a flow rate of 0.3 ml per
minute. Detection of CGA-152005 is performed using an ultraviolet
detector at a wavelength of 225 nm. The injection volume is 10 μl. The
HPLC conditions are listed in Table 1.

2.0 Standardization

Standardize the HPLC system by injecting 10-μl aliquots of
standard solutions of CGA-152005 in a working range of 0.6-10
ng/injection. Generate a linear
regression from the data by
comparing detector response and ng
injected. See section I.1.0 for
preparation of analytical
standards.

E. INTERFERENCES

None.

F. CONFIRMATORY TECHNIQUES

None.

G. TIME REQUIRED

The extraction and cleanup of a set of 6
samples may be completed within a time
period of 8 hours. HPLC analysis can be
performed overnight using automatic
injection.

H. MODIFICATIONS AND POTENTIAL PROBLEMS

1.0 Emulsion Formation

Some samples may develop emulsions
during partitioning (during steps
in sections C.2.2 and C.2.4).
These may be cleared by allowing to
settle out and then gently shaken
with a glass rod. Slight heating
may help (a hair dryer was used),
but care must be taken to avoid
temperatures in excess of 40°C.
Any emulsions that remain should
stay with the aqueous phases during
the partitioning steps.
2.0 Heat

During the evaporation of the solvents from the samples in sections C.2.1, C.3.2, and C.3.4, any water bath used must not have a temperature >40°C and the samples should be removed just as soon as they reach dryness. A temperature of 35°C is required for removal of the solvent from the Alumina-A eluate. Excessive temperature, especially when the sample has gone to dryness, may lead to analyte decomposition.

3.0 Degradation in Methanol

After elution from the Alumina-A column, the solvent must be evaporated as soon as possible, since CGA-152005 was reported to be unstable in methanol. Do not store the sample overnight in the methanol:acetonitrile eluent.

I. PREPARATION OF STANDARD SOLUTIONS

1.0 Preparation of Analytical Standards

Weigh 10 mg of CGA-152005 analytical standard into a 100-ml volumetric flask and dilute to the mark with acetonitrile. Make serial dilutions of the 0.1 mg/ml standard solution with 50% acetonitrile: 50% picopure water to give a series of fortification/analytical standards in a range of 0.06 µg/ml to 1.0 µg/ml of CGA-152005. Store the standard solutions in amber bottles at 4°C in the dark when not in use.

2.0 Preparation of Fortification Standards

The 1.0 µg/ml standard used to fortify standards is prepared in section I.1.0 above. See section K.1.0 for fortification procedures.
J. METHODS OF CALCULATION

1.0 Linear Regression Analysis

Inject 10-μl aliquots of sample extracts onto the HPLC system. Compare the analyte peak height found in the sample extracts to the peak heights determined for the analytical standards by entering them into a linear regression program or by using a computer system (e.g., VG Multichrom) to determine the nanograms of CGA-152005 in the injected aliquot.

2.0 Calculation of Sample Concentration

Calculate the residue results in terms of ppm of CGA-152005 by using the following equation:

\[
1 \text{ ppm} = \frac{(\text{ng CGA-152005 Found})}{(\text{mg sample injected}) (\%R)}
\]

Where the ng CGA-152005 is obtained from the linear regression analysis in section J.1.0 and the mg sample injected is calculated as follows: (Equation 2)

\[
2 \text{ mg inj.} = \frac{(G)(V_a)}{(V_e)(V_f)}
\]

\[ G = \text{milligrams sample extracted} \]
\[ V_a = \text{aliquot volume (from section C.1.1)} \]
\[ V_e = \text{extraction volume (from section C.1.1)} \]
\[ V_f = 180 \text{ ml (or 90 ml for milk)} + \]
\[ \text{(sample size in grams)} \]
\[ \text{(sample % moisture expressed as a decimal)} \]

The % moisture for each substrate used in this work was 88% for tissues, 0% for fat, 90% for blood, 87% for milk, 80% for whole egg, and 48% for egg yolks.
\[ V_i = \text{injection volume (\mu l)} \]
\[ V_f = \text{total volume of final injection solution (\mu l)} \]

The %R for the fresh fortifications shown in equation (1) is used as a correction for procedural recoveries, expressed as a decimal, and is shown in equation 3 (section K.2.0).

K. **Fortification Experiments**

1.0 **Substrate Fortification**

This method is validated for each set of samples analyzed by including an untreated control sample and one or more control samples fortified immediately prior to extraction with CGA-152005. For example, add 0.5 ml of a 1.0 \( \mu \)g/ml standard solution of CGA-152005 to 10 g of control tissues or 50 g of milk for a 0.05 ppm or 0.01 ppm fortification, respectively. The volume of the various fortification standards used can be varied from 0.5 to 2.0 ml depending on the desired fortification level. Allow the solvent to evaporate for at least 20 minutes prior to addition of extraction solvent. Analyze control and freshly fortified samples along with the treated samples according to the procedures of the method.

2.0 **Calculation of Procedural Recovery**

The final ppm value of the control and fortified samples can be obtained using the calculations shown in section J.2.0. Determine the % recovery by first subtracting the background detector response, if any, in the control sample from the CGA-152005 response in the recovery sample. Calculate the
procedural recovery factor as a percentage (R) by the equation:

\[
(3) \quad \text{R} = \frac{\text{ppm CGA-152005 found}}{\text{ppm CGA-152005 added}} \times 100
\]

III. RESULTS AND DISCUSSION

The objective of Protocol 26-92¹ was to validate analytical Method AG-592 for the quantification of residues of CGA-152005 in meat, milk, eggs, and blood. This was accomplished, as demonstrated by the results in this report, by analyzing control and CGA-152005 fortified control samples of poultry liver, peritoneal fat, lean meat, and eggs, and beef liver, kidney, tenderloin, round, omental fat, perirenal fat, blood, and dairy milk, and goat liver, omental fat, round, kidney, and milk. In addition, samples of poultry lean meat and eggs and goat liver, omental fat, round, kidney, and milk, obtained from metabolism studies²,³ in which the animals had been dosed with radiolabelled CGA-152005, were analyzed. The screening level for all the substrates is 0.050 ppm except milk which has a screening level of 0.01 ppm. The limit of detection, defined as the lowest concentration standard injected during a run, is 0.60 ng CGA-152005. The limit of determination (screening level), defined by the lowest fresh fortification level within a run, is 0.05 ppm for all substrates except milk which is 0.01 ppm.

Typical standard chromatograms of CGA-152005 at various concentrations are shown in Figure 3 and were obtained during the analysis of poultry lean meat. The concentrations of CGA-152005 and their respective peak heights from this run are shown in Table II. The calibration plot for this run is shown in Figure 4 and the correlation coefficient of 0.99938 is typical of the calibration plots obtained during this study. The peak heights obtained for the analytical standards were reproducible over the course of the study when injected under similar chromatographic conditions.
conditions, thus demonstrating the stability of the injection standards.

Reagent blanks were also analyzed during the analysis of poultry liver (representative of tissues), poultry peritoneal fat (representative of fat samples), beef tenderloin (representative of lean meats), and beef blood (representative of high moisture substrates). The chromatograms of the reagent blanks are shown in Figure 5. As can be seen, there are no peaks near the retention time (about 22 minutes) of CGA-152005 in any of the chromatograms.

A. ACCURACY

Table III shows the results of analyzing control and freshly fortified control samples. The recoveries for all samples at all fresh fortification levels ranged from 70 to 111% except for the single 0.50 ppm fortification level for beef blood which was 58%. The mean recoveries for each substrate ranged from 75% (SD=4.0) for goat omental fat to 104% (SD=3.8 and 5.4, respectively) for goat liver and beef liver. The average recovery for all substrates was 90% with a standard deviation of 11% over a CGA-152005 fortification range of 0.01 to 5.0 ppm and the control samples showed no background residues at or above the limit of determination of the method in any substrate. These recovery results demonstrate the accuracy of the method. Representative chromatograms for all the substrates listed in Table III are shown in Figures 6 through 22.

B. PRECISION

The results of subjecting the \(^{14}C\)-CGA-152005 containing substrates to analysis using Method AG-592 are shown in Table IV. The narrow range and relatively small standard deviations of the ppm found for each substrate demonstrate the precision of the method.
C. EXTRACTABILITY AND ACCOUNTABILITY

The $^{14}$C analyses were performed by transferring an aliquot portion of the appropriate sample (extract or final fraction) to a scintillation vial, adding 15 ml of Beckman Ready-Safe liquid scintillation cocktail, followed by measurement on a Beckman LS 7800 liquid scintillation counting (LSC) instrument. The results are obtained in units of disintegrations per minute (dpm) per volume of sample measured. The % extractabilities were calculated from data obtained by subjecting an aliquot portion of the sample extract to LSC, converting the dpm/ml data to parts per million (ppm), and comparing the results (by percentages) to the total $^{14}$C residue in the sample, as determined by combustion analysis (as reported in the metabolism studies$^{2,3}$). The % accountabilities of Method AG-592 were calculated from data obtained by the method (liquid chromatography analysis), compared (by percentages) to the total $^{14}$C residue in the sample. The following calculation was performed to convert the dpm/ml value obtained by LSC from one of the poultry lean meat extracts into units of ppm. The activity of the $^{14}$C-CGA-152005 fed to the animal was 40.2 μCi/mg. A 10 gram sample was extracted with 180 ml 90% acetone/10% 0.1% sodium bicarbonate and 3 ml of the extract contained 12,219 dpm. Therefore,

$$\left( \frac{12,219 \text{ dpm}}{3 \text{ ml}} \right) \left( \frac{1,000 \mu\text{g}}{\text{mg}} \right) \left( \frac{\text{mg}}{40.2 \mu\text{Ci}} \right) \left( \frac{1 \times 10^6 \mu\text{Ci}}{\text{Cl}} \right) \left( \frac{\text{Cl}}{3.7 \times 10^6 \text{ dpm}} \right) \left( \frac{\text{dps}}{60 \text{ dpm/}} \right) \left( \frac{180 \text{ ml}}{10 \text{ g}} \right)$$

$$= 0.82 \mu\text{g} \text{ or ppm}$$

The extractability and accountability data are shown in Table V. The % extractabilities ranged from a low of 72% for eggs to a high of 148% for goat omental fat. The accountabilities ranged from a low of 28% for eggs to a high of
142% for goat omental fat. It should be noted that these ¹⁴C-CGA-152005 containing substrates were obtained from metabolism studies²,³ which are still in progress and that the combustion results measure only the total ¹⁴C radioactivity in the sample. Therefore, the lower accountabilities may result from a decrease in the quantity of CGA-152005 in these samples due to metabolism to other compounds. The results obtained by liquid chromatographic analysis using method AG-592 are in reasonably good agreement with the results obtained from the LSC measurements of the same final fractions as shown in columns G and I of Table V. Representative chromatograms obtained by subjecting the ¹⁴C-CGA-152005 containing substrates to the analytical procedures outlined in Method AG-592 are shown in Figures 6 through 12.

Test substance ID, test system ID, protocol amendments, protocol deviations, and circumstances affecting the quality and integrity of the data are also reported in Residue Test Report RI-MV-001-92⁴. All raw data associated with this study and the original final AG-592 method and protocol are archived in the Residue Chemistry Archives at CIBA-GEIGY Corporation, Greensboro, NC. No specimens remain to be retained.

IV. CONCLUSION

Method AG-592 is a valid and accurate method for the determination of CGA-152005 in meat, milk, eggs, and blood. This conclusion is based on the accuracy, precision, extractability, and accountability of the method as demonstrated by the results in this study.
V. CERTIFICATION

The reports and experimental results included in this study, Laboratory Project I.D. AG-592, are certified to be authentic accounts of the experiments.

[Signature]
Robert A. Yokley, Ph.D.
Method Development
Residue Chemistry Department
919-632-2295

3-31-92
Date

CERTIFICATION OF GOOD LABORATORY PRACTICES

The analytical work reported in AG-592 was performed in accordance with Good Laboratory Practice Standards, 40 CFR Part 160.

[Signature]
Robert A. Yokley, Ph.D.
Study Director

3-31-92
Date

[Signature]
Robert K. Williams, Manager
Method Development
Residue Chemistry Department
Submitter/Sponsor

7/31/92
Date
VI. QUALITY ASSURANCE STATEMENT

Method Title: ANALYTICAL METHOD FOR THE DETERMINATION OF CGA-152005 IN MEAT, MILK, BLOOD, AND EGGS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Study Director: R. Yokley
Protocol Number: 26-92

Project Number: 168982
Final Report No.: AG-592

Pursuant to Good Laboratory Practice Regulations, this statement verifies that the aforementioned study was inspected and/or audited and the findings reported to Management and to the Study Director(s) by the Quality Assurance Unit on the dates listed below.

<table>
<thead>
<tr>
<th>Audit Type</th>
<th>Inspection/Audit Date</th>
<th>Reporting Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocol</td>
<td>2/07/92</td>
<td>2/07/92</td>
</tr>
<tr>
<td>In-Progress</td>
<td>2/28/92</td>
<td>3/02/92</td>
</tr>
<tr>
<td>Final Report</td>
<td>3/26, 27, 30/92</td>
<td>3/30/92</td>
</tr>
</tbody>
</table>

Prepared by: Lynda A. Sayers
Date: 3/30/92
VII. LIST OF TABLES AND FIGURES

TABLE I. LIQUID CHROMATOGRAPHIC OPERATING PARAMETERS FOR THE ANALYSIS OF CGA-152005

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument:</td>
<td>Perkin-Elmer Model Series-4 Solvent Delivery System or equivalent.</td>
</tr>
<tr>
<td></td>
<td>Perkin-Elmer Model ISS-100 Automatic HPLC sampler or equivalent.</td>
</tr>
<tr>
<td>Column Oven:</td>
<td>BioRad HPLC column heater, model number 125-0425</td>
</tr>
<tr>
<td>Oven Temp.:</td>
<td>30°C</td>
</tr>
<tr>
<td>Column:</td>
<td>Supelcosil LC-18-DB, 250mm x 2.1 mm, 5 μm particle size (Supelco cat.</td>
</tr>
<tr>
<td></td>
<td>#5-7940) with Supelguard LC-18-DB guard column.</td>
</tr>
<tr>
<td>Mobile Phase:</td>
<td>60% 0.05% phosphoric acid in picopure water: 40% acetonitrile</td>
</tr>
<tr>
<td>Retention Time:</td>
<td>~22 minutes</td>
</tr>
<tr>
<td>Detection:</td>
<td>ABI Kratos Spectroflow Model 783 Programmable Absorbance Detector or</td>
</tr>
<tr>
<td></td>
<td>equivalent variable wavelength detector.</td>
</tr>
<tr>
<td>Wavelength:</td>
<td>225 nm</td>
</tr>
<tr>
<td>Attenuation:</td>
<td>0.006 AUFs</td>
</tr>
<tr>
<td>Flow Rate:</td>
<td>0.3 ml/min</td>
</tr>
<tr>
<td>Volume Injected:</td>
<td>10 μl</td>
</tr>
<tr>
<td>Chart Speed:</td>
<td>0.25 cm/min</td>
</tr>
<tr>
<td>Run Time:</td>
<td>30 min/injection</td>
</tr>
<tr>
<td>Data Acquisition:</td>
<td>Microvax II (Bones or Q) Operating System, VMS Version 5.3-1 Application Software:</td>
</tr>
<tr>
<td></td>
<td>VG Multichrom Version 1.8 Worksheet Version: Ws.pas 1.3.1</td>
</tr>
</tbody>
</table>
TABLE II. **TYPICAL STANDARDIZATION DATA FOR CGA-152005 (FROM THE ANALYSIS OF POULTRY LEAN MEAT). SEE ALSO FIGURES 3 AND 4**

<table>
<thead>
<tr>
<th>CGA-152005 ng Injected</th>
<th>Peak Height (μV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.60</td>
<td>102</td>
</tr>
<tr>
<td>1.0</td>
<td>204</td>
</tr>
<tr>
<td>2.0</td>
<td>404</td>
</tr>
<tr>
<td>4.0</td>
<td>831</td>
</tr>
<tr>
<td>6.0</td>
<td>1224</td>
</tr>
<tr>
<td>10.0</td>
<td>2175</td>
</tr>
</tbody>
</table>

Correlation Coefficient: 0.99938
Slope: 217.9
Intercept: -33.85
### TABLE III. **RECOVERY RESULTS FOR CONTROL AND CGA-152005 FORTIFIED CONTROL SUBSTRATES USING METHOD AG-592**

<table>
<thead>
<tr>
<th>(1) SUBSTRATE</th>
<th>PROTOCOL 26-92 SAMPLE #</th>
<th>CGA-152005 FORTIFICATION (ppm)</th>
<th>CGA-152005 (ppm)</th>
<th>(2) % RECOVERY (AG-592)</th>
<th>--- % RECOVERY STANDARD MEAN DEVIATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poultry</td>
<td>02</td>
<td>0</td>
<td>&lt;0.05</td>
<td>103</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>03</td>
<td>0.05</td>
<td>0.051</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>04</td>
<td>0.05</td>
<td>0.048</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>05</td>
<td>0.20</td>
<td>0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>06</td>
<td>0.50</td>
<td>0.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poultry</td>
<td>08</td>
<td>0</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peritoneal</td>
<td>09</td>
<td>0.05</td>
<td>0.044</td>
<td>89</td>
<td>88</td>
</tr>
<tr>
<td>Fat</td>
<td>10</td>
<td>0.05</td>
<td>0.045</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>0.20</td>
<td>0.18</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.50</td>
<td>0.42</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>Beef</td>
<td>13</td>
<td>0</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>14</td>
<td>0.05</td>
<td>0.053</td>
<td>106</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0.05</td>
<td>0.055</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>0.20</td>
<td>0.20</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>0.50</td>
<td>0.49</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>Beef</td>
<td>18</td>
<td>0</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>19</td>
<td>0.05</td>
<td>0.047</td>
<td>83</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.05</td>
<td>0.050</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>0.20</td>
<td>0.19</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>0.50</td>
<td>0.50</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>Beef</td>
<td>24</td>
<td>0</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tenderloun</td>
<td>25</td>
<td>0.05</td>
<td>0.047</td>
<td>93</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>0.05</td>
<td>0.047</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>0.20</td>
<td>0.22</td>
<td>108</td>
<td></td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>0.50</td>
<td>0.48</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>Beef Round</td>
<td>29</td>
<td>0</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.05</td>
<td>0.046</td>
<td>91</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>0.05</td>
<td>0.042</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>0.20</td>
<td>0.139</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>0.50</td>
<td>0.363</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>Beef</td>
<td>34</td>
<td>0</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omental Fat</td>
<td>35</td>
<td>0.05</td>
<td>0.041</td>
<td>82</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>0.05</td>
<td>0.049</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>0.20</td>
<td>0.16</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>0.50</td>
<td>0.46</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>Beef</td>
<td>39</td>
<td>0</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penntenal Fat</td>
<td>40</td>
<td>0.05</td>
<td>0.040</td>
<td>79</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>41</td>
<td>0.05</td>
<td>0.047</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>0.20</td>
<td>0.17</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>0.50</td>
<td>0.45</td>
<td>90</td>
<td></td>
</tr>
</tbody>
</table>

(1) All samples are from Test Number RJ-MV-001-92 - overall mean of 90% with a SD of 11% (n=61).  
(2) Recovery samples were corrected for control residues where present.
### TABLE III. RECOVERY RESULTS FOR CONTROL AND CGA-152005 FORTIFIED CONTROL SUBSTRATES USING METHOD AG-592 (Continued)

<table>
<thead>
<tr>
<th>(1) SUBSTRATE</th>
<th>PROTOCOL 26-92 SAMPLE #</th>
<th>CGA-152005 FORTIFICATION (ppm)</th>
<th>CGA-152005 (ppm)</th>
<th>(2) % RECOVERY (AG-592)</th>
<th>% RECOVERY STANDARD MEAN DEVIATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef Blood</td>
<td>45 0</td>
<td>&lt;0.05</td>
<td>0.046</td>
<td>93</td>
<td>78 16</td>
</tr>
<tr>
<td></td>
<td>46 0.05</td>
<td></td>
<td>0.044</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td></td>
<td>47 0.05</td>
<td></td>
<td>0.15</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48 0.20</td>
<td></td>
<td>0.29</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td></td>
<td>49 0.50</td>
<td></td>
<td>&lt;0.01</td>
<td>82</td>
<td>86 8.2</td>
</tr>
<tr>
<td>Beef Milk</td>
<td>50 0</td>
<td></td>
<td>0.0082</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td></td>
<td>51 0.01</td>
<td></td>
<td>0.010</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td></td>
<td>52 0.01</td>
<td></td>
<td>0.084</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td></td>
<td>53 0.10</td>
<td></td>
<td>0.393</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>Poultry</td>
<td>55 0</td>
<td>&lt;0.05</td>
<td>0.049</td>
<td>97</td>
<td>97 5.1</td>
</tr>
<tr>
<td>Lean Meat</td>
<td>56 0.05</td>
<td></td>
<td>0.051</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td></td>
<td>57 0.05</td>
<td></td>
<td>0.46</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>Eggs</td>
<td>62 0</td>
<td>&lt;0.05</td>
<td>0.045</td>
<td>89</td>
<td>87 2.3</td>
</tr>
<tr>
<td></td>
<td>63 0.05</td>
<td></td>
<td>0.042</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td></td>
<td>64 0.50</td>
<td></td>
<td>0.43</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>Goat Liver</td>
<td>69 0</td>
<td>&lt;0.05</td>
<td>0.054</td>
<td>108</td>
<td>104 3.8</td>
</tr>
<tr>
<td></td>
<td>70 0.05</td>
<td></td>
<td>0.051</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td></td>
<td>71 0.05</td>
<td></td>
<td>1.02</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>Goat</td>
<td>72 1.00</td>
<td></td>
<td>&lt;0.05</td>
<td>72</td>
<td>75 4.0</td>
</tr>
<tr>
<td>Omental Fat</td>
<td>77 0.05</td>
<td></td>
<td>0.036</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td></td>
<td>78 0.05</td>
<td></td>
<td>0.079</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>Goat Round</td>
<td>83 0</td>
<td>&lt;0.05</td>
<td>0.051</td>
<td>101</td>
<td>100 2.3</td>
</tr>
<tr>
<td></td>
<td>84 0.05</td>
<td></td>
<td>0.051</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td></td>
<td>85 0.05</td>
<td></td>
<td>0.19</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>Goat Kidney</td>
<td>90 0</td>
<td>&lt;0.05</td>
<td>0.050</td>
<td>100</td>
<td>102 3.8</td>
</tr>
<tr>
<td></td>
<td>91 0.05</td>
<td></td>
<td>0.033</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td></td>
<td>92 0.05</td>
<td></td>
<td>5.0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Goat Milk</td>
<td>93 0.05</td>
<td></td>
<td>&lt;0.05</td>
<td>87</td>
<td>86 15</td>
</tr>
<tr>
<td></td>
<td>98 0.05</td>
<td></td>
<td>0.043</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>99 0.05</td>
<td></td>
<td>0.071</td>
<td>71</td>
<td></td>
</tr>
</tbody>
</table>

(1) All samples are from Test Number RU-MV-001-92 - overall mean of 90% with a SD of 11% (n=61)
(2) Recovery samples were corrected for control residues where present.
TABLE IV.  PRECISION OF ANALYTICAL METHOD AG-592 AS DEMONSTRATED BY THE RESULTS OF ANALYZING ¹³C-GGA-152005 CONTAINING SUBSTRATES

<table>
<thead>
<tr>
<th>SUBSTRATE</th>
<th>PROTOCOL 26-92 SAMPLE #</th>
<th>(I) FOUND BY AG-592 (ppm)</th>
<th>MEAN (ppm)</th>
<th>RANGE (ppm)</th>
<th>STANDARD DEVIATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poultry Lean Meat</td>
<td>59, 60, 61</td>
<td>0.64, 0.62, 0.65</td>
<td>0.63</td>
<td>0.62-0.65</td>
<td>0.015</td>
</tr>
<tr>
<td>Eggs</td>
<td>66, 67, 68</td>
<td>0.099, 0.087, 0.088</td>
<td>0.091</td>
<td>0.087-0.099</td>
<td>0.006</td>
</tr>
<tr>
<td>Goat Liver</td>
<td>73, 74, 75</td>
<td>1.37, 1.44, 1.42</td>
<td>1.41</td>
<td>1.37-1.44</td>
<td>0.037</td>
</tr>
<tr>
<td>Goat Omental Fat</td>
<td>80, 81, 82</td>
<td>0.082, 0.081, 0.081</td>
<td>0.082</td>
<td>0.081-0.084</td>
<td>0.002</td>
</tr>
<tr>
<td>Goat Round</td>
<td>87, 88, 89</td>
<td>0.21, 0.23, 0.22</td>
<td>0.22</td>
<td>0.21-0.23</td>
<td>0.008</td>
</tr>
<tr>
<td>Goat Kidney</td>
<td>94, 95, 96</td>
<td>5.4, 5.3, 5.3</td>
<td>5.4</td>
<td>5.3-5.5</td>
<td>0.053</td>
</tr>
<tr>
<td>Goat Milk</td>
<td>101, 102, 103</td>
<td>0.055, 0.052, 0.037</td>
<td>0.048</td>
<td>0.037-0.055</td>
<td>0.010</td>
</tr>
</tbody>
</table>

(1) Corrected by % recovery from fresh fortification as shown by Table III for the fresh fortification concentration nearest that of the total ¹³C residues.
<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SUBSTRATE</td>
<td>TOTAL RESIDUE</td>
<td>ppm</td>
<td>% EXT</td>
<td>FINAL FRACTION 14C</td>
<td>14C FRACTION CORRECTED</td>
<td>FINAL FRACTION</td>
<td>I-FINAL FRACTION CORRECTED</td>
<td>ACCOUNTABILITY %</td>
</tr>
<tr>
<td></td>
<td>SAMPLE CODE</td>
<td></td>
<td></td>
<td></td>
<td>(ppm)</td>
<td>(ppm)</td>
<td>(ppm)</td>
<td>(ppm)</td>
<td>(%)</td>
</tr>
<tr>
<td>Poultry Lean Meat</td>
<td>H91260507</td>
<td>0.705</td>
<td>0.82, 0.82, 0.69</td>
<td>110</td>
<td>0.55, 0.53, 0.55</td>
<td>0.60, 0.58, 0.60</td>
<td>0.58, 0.56, 0.59</td>
<td>0.64, 0.62, 0.65</td>
<td>90</td>
</tr>
<tr>
<td>Eggs</td>
<td>H91260533</td>
<td>0.326</td>
<td>0.24, 0.21, 0.24</td>
<td>72</td>
<td>0.083, 0.081, 0.082</td>
<td>0.096, 0.093, 0.095</td>
<td>0.085, 0.075, 0.076</td>
<td>0.099, 0.087, 0.088</td>
<td>28</td>
</tr>
<tr>
<td>Goat Liver</td>
<td>G90480033</td>
<td>1.371</td>
<td>1.44, 1.38, 1.53</td>
<td>106</td>
<td>1.17, 1.21, 1.22</td>
<td>1.17, 1.21, 1.22</td>
<td>1.37, 1.44, 1.42</td>
<td>1.37, 1.44, 1.42</td>
<td>103</td>
</tr>
<tr>
<td>Goat Ommental Fat</td>
<td>G90480024</td>
<td>0.058</td>
<td>0.080, 0.084, 0.090</td>
<td>116</td>
<td>0.064, 0.062, 0.068</td>
<td>0.080, 0.078, 0.086</td>
<td>0.065, 0.064, 0.067</td>
<td>0.082, 0.081, 0.084</td>
<td>142</td>
</tr>
<tr>
<td>Goat Round</td>
<td>G904800031</td>
<td>0.209</td>
<td>0.25, 0.24, 0.24</td>
<td>116</td>
<td>0.20, 0.20, 0.20</td>
<td>0.20, 0.21, 0.21</td>
<td>0.21, 0.22, 0.22</td>
<td>0.21, 0.23, 0.22</td>
<td>106</td>
</tr>
<tr>
<td>Goat Kidney</td>
<td>G90480026</td>
<td>0.048</td>
<td>4.7, 5.2, 5.6</td>
<td>103</td>
<td>4.7, 4.7, 4.6</td>
<td>4.7, 4.7, 4.6</td>
<td>5.4, 5.5, 5.3</td>
<td>5.4, 5.5, 5.3</td>
<td>107</td>
</tr>
<tr>
<td>Goat Milk</td>
<td>G90480012</td>
<td>0.069</td>
<td>0.034, 0.035, 0.035</td>
<td>79</td>
<td>0.023, 0.025, 0.015</td>
<td>0.035, 0.036, 0.022</td>
<td>0.039, 0.037, 0.026</td>
<td>0.055, 0.052, 0.037</td>
<td>70</td>
</tr>
</tbody>
</table>

(1) As determined by combustion analysis in metabolism studies.\(^2,3\)
(2) Determined by liquid scintillation counting of aliquots of the extract from Section II C.1 of AG-592
(3) Average of three extractions shown in column D divided by the total residue shown in Column C, times 100.
(4) Determined by liquid scintillation counting of aliquots of the final fraction from Section II C.3.5
(5) Corrected by % Recovery from fresh fortification as shown in Table III for the fresh fortification concentration nearest that of the total \(^{14}\)C residue
(6) Determined by high performance liquid chromatography.
(7) Average of Column I, divided by Column C, and multiplied by 100
FIGURE 1. STRUCTURE AND CHEMICAL NAME OF CGA-152005

N-[[4-Methoxy-6-methyl-1,3,5-triazin-2-yl]amino] carbonyl]-2-(3,3,3-trifluoropropyl)-benzenesulfonamide
FIGURE 2. ANALYTICAL PROCEDURE FLOWCHART FOR AG-592

10 g. sample for tissues, blood or egg. 50 g. sample for milk.

Homogenize with 90 ml 90% acetone:10% 0.1% sodium bicarbonate, 30 seconds.

Filter (R&A #802 filter)

Place filter with residual tissues into bottle and homogenize again with 90 ml 90% acetone:10% 0.1% sodium bicarbonate. Refilter (R&A #802) and combine extracts.

Measure volume and remove acetone via rotary evaporation (38-40°C)

Add 25 ml 0.4% sodium carbonate. Partition (2 x 30 ml) with methyl tert-butyl ether:hexane (1:1). Discard organic.

(aqueous)

Add 20 ml 0.8% phosphoric acid and 20 ml water saturated with sodium chloride. Partition 3 x 60 ml with dichloromethane:hexane (1:1). Discard aqueous.

(organic)

Dry with sodium sulfate and reduce to 10 ml via rotary evaporation (35°C).

Alumina A Sep-Pak Solid Phase Extraction

Condition column with 25 ml methanol:acetonitrile (1:1), 15 ml ethyl acetate, and 25 ml dichloromethane:hexane (1:1)

Add sample (quantitatively)

Wash column with 15 ml hexane (discard wash)

Wash column with 25 ml ethyl acetate (discard wash)

Wash column with 15 ml 5% acetonitrile in ethyl acetate (discard wash)

Elute sample with 30 ml methanol:acetonitrile (60:40). Collect in 100-ml round bottom flask.

Evaporate to just dryness with rotovap (35°C)

Add 1 ml acetonitrile. Sonicate and swirl.

Add 1 ml picopure water. Sonicate and swirl. Filter through Acrodisc LC PVDF 25 mm diameter, 0.45 um pore filter.

Rinse round bottom and filter with 1 ml acetonitrile:water (1:1)

HPLC (Supelcosil LC-DB-18, 25cm x 2.1 mm; mobile phase = 60% 0.05% phosphoric acid in picopure water:40% acetonitrile; UV detection at 225 nm)
Figure 3. Typical Standard Chromatograms (from Poultry Lean Meat Analyses).

Quantity Injected in ng LCGC-152005 (1) 6.0 (2) 1.0 (3) 2.0
(4) 4.0 (5) 6.0 (6) 10.0.
C-G Multichrom 1.8 (BONES)

Calibration Name: 27 VALMAR12.

Peak: cgals2005

Calibration level plot

FIGURE 4. CALIBRATION PLOT OF THE STANDARDS OBTAINED FROM THE ANALYSIS OF POULTRY LEAN MEAT

Constant: -3.385802E+1
1st degree: 2.17901E+2

Curve fit: Linear
Correlation coefficient: 0.99938
Standard error: 3.08029E+1

Reported on 13-MAR-1992 at 07:17
Figure 5. Chromatograms Of The Reagent Blanks Obtained During The Analyses Of (1) Poultry Liver, (2) Poultry Peritoneal Fat, (3) Beef Tendrin, and (4) Beef Blood.

1. 28.77 mg equivalent injected, 0.00 ng CDA-150005, 0.050 ppm
2. 28.77 mg equivalent injected, 0.00 ng CDA-150005, 0.050 ppm
3. 29.30 mg equivalent injected, 0.00 ng CDA-150005, 0.050 ppm
4. 28.42 mg equivalent injected, 0.00 ng CDA-150005, 0.050 ppm
Figure 6. Representative Chromatograms From The Analysis Of Radiolabelled CGA-152005 Containing Poultry Lean Meat Using Method AG-592.

1. Control. 27.31 mg injected, 0.60 ng CGA-152005, 0.050 ppm found
2. 0.050 ppm fortification. 27.31 mg injected, 1.33 ng CGA-152005, 0.049 ppm found, 97 % Recovery.
3. 0.50 ppm fortification. 8.30 mg injected, 3.79 ng CGA-152005, 0.46 ppm found, 91 % Recovery.
4. 0.71 ppm as 14C-CGA-152005. 6.81 mg injected, 3.97 ng CGA-152005, 0.58 ppm found, 83 % Accountability.
Figure 7. Representative Chromatograms From The Analysis Of Radiolabelled CGA-152005 Containing Eggs Using Method AG-592.

1. Control. 20.37 mg injected. 0.66 ng CGA-152005. 0.050 ppm
2. 0.050 ppm fortification. 28.37 mg injected. 1.27 ng CGA-152005. 0.045 ppm found. 89% Recovery
3. 0.50 ppm fortification. 8.51 mg injected. 3.67 ng CGA-152005. 0.43 ppm found. 86% Recovery
4. 0.326 ppm as 14C-CGA-152005. 14.35 mg injected. 1.22 ng CGA-152005. 0.085 ppm found. 26% Accountability.
Figure 8. Representative Chromatograms From The Analysis Of Radiolabelled CGA-152005 Containing Goat Liver Using Method AG-592.

1. Control, 27.31 mg injected, 0.60 ng CGA-152005, 0.050 ppm
2. 0.050 ppm fortification, 27.31 mg injected, 1.48 ng CGA-152005, 0.054 ppm found, 108 % Recovery.
3. 1.00 ppm fortification, 4.12 mg injected, 4.21 ng CGA-152005, 1.02 ppm found, 102 % Recovery.
4. 1.37 ppm as 14C-CGA-152005, 4.20 mg injected, 5.76 ng CGA-152005, 1.37 ppm found, 100% Accountability.
Figure 9. Representative Chromatograms From The Analysis Of Radio-labelled CGA-152005 Containing Omental Fat Using Method AG-592.

Control: 0.05 ppm as CGA-152005 injected; 0.04 ppm found. 77% Recovery.

D. 0.06 ppm as CGA-152005 injected; 0.06 ppm found. 78% Recovery.

E. 0.055 ppm as CGA-152005 injected; 0.055 ppm found. 75% Recovery.

F. 0.07 ppm as CGA-152005 injected; 0.07 ppm found. 72% Recovery.
Figure 10. Representative Chromatograms From The Analysis Of Radiolabelled CGA-152005 Containing Goat Round Using Method AG-592.

1. Control, 26.95 mg injected, 0.60 ng CGA-152005, 0.050 ppm
2. 0.050 ppm fortification, 26.95 mg injected, 1.34 ng CGA-152005, 0.051 ppm found, 101% Recovery.
3. 0.20 ppm fortification, 26.95 mg injected, 5.23 ng CGA-152005, 0.19 ppm found, 97% Recovery.
4. 0.21 ppm 14C-CGA-152005, 26.95 mg injected, 5.66 ng CGA-152005, 0.21 ppm found, 99% Accountability.
Figure 11. Representative Chromatograms From The Analysis Of Radiolabelled CGA-152005 Containing Goat Kidney Using Method AG-592.

1. Control. 27.31 μg injected, 0.60 ng CGA-152005, 0.050 ppm
2. 0.050 ppm fortification. 27.36 μg injected, 1.38 ng CGA-152005, 0.050 ppm found, 100% Recovery.
3. 5.00 ppm fortification. 0.82 ng injected, 4.16 ng CGA-152005, 5.02 ppm found, 100% Recovery.
4. 5.05 ppm as 14C-CGA-152005, 0.84 ng injected, 4.55 ng CGA-152005, 5.41 ppm found, 107% Accountability.
Figure 12. Representative Chromatograms From The Analysis Of Radiolabelled CGA-152005 Containing Goat Milk Using Method AG-592.

1. Control, 112.36 mg injected, 10.60 ng CGA-152005, 10.850 ppm
2. 0.050 ppm fortification, 112.36 mg injected, 6.87 ng CGA-152005, 0.043 ppm found, 87% Recovery.
3. 0.10 ppm fortification, 33.71 mg injected, 2.38 ng CGA-152005, 0.071 ppm found, 71% Recovery.
4. 0.009 ppm as 14C-CGA-152005, 33.71 mg injected, 1.32 ng CGA-152005, 0.039 ppm found, 57% Accountability.
Figure 13. Representative Chromatograms From The Analysis Of Poultry Liver For CGA-152005 Using Method AG-592.

1. Control, 28.01 mg injected, 0.60 ng CGA-152005, 0.050 ppm
2. 0.050 ppm fortification, 27.85 mg injected, 1.42 ng CGA-152005, 0.051 ppm found, 103% Recovery.
3. 0.20 ppm fortification, 28.19 mg injected, 5.61 ng CGA-152005, 0.20 ppm found, 99% Recovery.
4. 0.50 ppm fortification, 8.40 mg injected, 4.05 ng CGA-152005, 0.48 ppm found, 96% Recovery.
Figure 14. Representative Chromatograms From The Analysis Of Poultry Peritoneal Fat For CGA-152005 Using Method AG-592.

1. Control, 28.89 mg injected, 0.60 ng CGA-152005, 0.050 ppm
2. 0.050 ppm fortification, 29.82 mg injected, 1.32 ng CGA-152005, 0.044 ppm found, 89 % Recovery.
3. 0.20 ppm fortification, 28.70 mg injected, 5.04 ng CGA-152005, 0.18 ppm found, 88 % Recovery.
4. 0.50 ppm fortification, 8.94 mg injected, 3.73 ng CGA-152005, 0.42 ppm found, 63 % Recovery.
Figure 15. Representative Chromatograms From The Analysis of Beef Liver For CGA-152005 Using Method AG-592.

1. Control, 28.95 μg injected, 10.00 ng CGA-152005, 40.050 ppm found
2. 0.050 ppm fortification, 27.66 ng injected, 1.47 ng CGA-152005, 0.053 ppm found, 106 % Recovery
3. 0.20 ppm fortification, 27.66 ng injected, 5.59 ng CGA-152005, 0.20 ppm found, 101 % Recovery
4. 0.50 ppm fortification, 8.09 ng injected, 3.99 ng CGA-152005, 0.59 ppm found, 99 % Recovery.
Figure 16. Representative Chromatograms From The Analysis Of Beef Kidney For CGA-152005 Using Method AG-592.

1. Control. 27.48 mg injected, 10.60 ng CGA-152005, 10.050 ppm
2. 0.050 ppm fortification, 27.66 mg injected, 1.29 ng CGA-152005, 0.042 ppm found, 83% Recovery.
3. 0.20 ppm fortification, 27.66 mg injected, 5.20 ng CGA-152005, 0.18 ppm found, 92% Recovery.
4. 0.50 ppm fortification, 8.40 mg injected, 4.18 ng CGA-152005, 0.49 ppm found, 98% Recovery.
Figure 17. Representative Chromatograms From The Analysis Of Beef Tenderloin For CGA-152005 Using Method AG-592.

1. Control. 28.01 mg injected, 0.60 ng CGA-152005, 10.050 ppm
2. 0.050 ppm fortification, 28.72 mg injected. 1.34 ng CGA-152005. 0.047 ppm found, 93 % Recovery.
3. 0.20 ppm fortification, 28.55 mg injected. 6.14 ng CGA-152005. 0.22 ppm found, 108 % Recovery.
4. 0.50 ppm fortification, 8.51 mg injected. 4.11 ng CGA-152005. 0.48 ppm found, 97 % Recovery.
Figure 18. Representative Chromatograms from the Analysis Of Beef Round For CGA-15205 Using Method AG-592.

1. Control, 27.13 mg injected, 70.00 mg CGA-15205s 0.050 ppm found 70 % Recovery.
2. 0.050 ppm fortification, 27.66 mg injected, 0.040 mg CGA-15205s 0.46 ppm found, 70 % Recovery.
3. 0.050 ppm fortification, 27.66 mg injected, 0.046 mg CGA-15205s 0.36 ppm found, 70 % Recovery.
4. 0.050 ppm fortification, 27.66 mg injected, 0.080 mg CGA-15205s 0.2 ppm found, 70 % Recovery.
Figure 19. Representative Chromatograms From The Analysis of Beef Omental Fat For CGA-152005 Using Method AG-592.

1. Control, 27.41 mg injected, 10.60 ng CGA-152005, 10.050 ppm
2. 0.050 ppm fortification, 27.41 mg injected, 1.13 ng CGA-152005, 0.041 ppm found, 82% Recovery.
3. 0.20 ppm fortification, 27.41 mg injected, 4.25 ng CGA-152005, 0.16 ppm found, 77% Recovery.
4. 0.50 ppm fortification, 8.56 mg injected, 3.90 ng CGA-152005, 0.46 ppm found, 91% Recovery.
Figure 20. Representative Chromatograms From The Analysis Of Beef Perirenal Fat For CGA-152005 Using Method AG-592.

1. Control, 27.78 mg injected, 10.60 ng CGA-152005, 0.059 ppm
2. 0.050 ppm fortification, 27.78 mg injected, 1.10 ng CGA-152005, 0.040 ppm found, 79% Recovery
3. 0.20 ppm fortification, 27.04 mg injected, 4.00 ng CGA-152005, 0.17 ppm found, 85% Recovery
4. 0.50 ppm fortification, 8.22 mg injected, 3.69 ng CGA-152005, 0.45 ppm found, 90% Recovery
Figure 21. Representative Chromatograms From The Analysis Of Beef Blood For CGA-152005 Using Method AG-592.

1. Control, 28.22 mg injected, 0.60 ng CGA-152005, 0.050 ppm
2. 0.050 ppm fortification, 28.22 mg injected, 1.31 ng CGA-152005, 0.046 ppm found, 93 % Recovery.
3. 0.20 ppm fortification, 26.60 mg injected, 4.21 ng CGA-152005, 0.15 ppm found, 74 % Recovery.
4. 0.50 ppm fortification, 8.67 mg injected, 2.44 ng CGA-152005, 0.29 ppm found, 58 % Recovery.
Figure 22. Representative Chromatograms From The Analysis Of Dairy Milk For CGA-152005 Using Method AG-592.

1. Control, 112.36 mg injected, 10.60 ng CGA-152005 10.010 ppm
2. 0.010 ppm fortification, 112.36 mg injected, 0.93 ng CGA-152005, 0.008 ppm found, 82% Recovery.
3. 0.10 ppm fortification, 29.72 mg injected, 2.49 ng CGA-152005, 0.084 ppm found, 84% Recovery.
4. 0.50 ppm fortification, 8.92 mg injected, 3.50 ng CGA-152005, 0.39 ppm found, 79% Recovery.
VIII. REFERENCES


APPENDIX I

RESIDUE CHEMISTRY DEPARTMENT PROTOCOL NUMBER 26-92
AND AMENDMENT 1

SUBMITTER/SPONSOR:
Ciba Plant Protection
Ciba-Geigy Corporation
Post Office Box 18300
Greensboro, NC 27410
AGRICULTURAL DIVISION
CIBA-GEIGY CORPORATION

RESIDUE CHEMISTRY DEPARTMENT
PROTOCOL 26-92

VALIDATION OF "DRAFT" ANALYTICAL METHOD AG-592 FOR THE
DETERMINATION OF CGA-152005 IN MEAT, MILK, BLOOD, AND
EGGS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Study/Project No.: 168982

STUDY DIRECTOR: Robert A. Yokley

TITLE: Project Scientist, Method Development

SIGNATURE: Robert A. Yokley

DATE: February 10, 1992

SPONSOR APPROVAL:

R. K. Williams

TITLE: Manager, Method Development

SIGNATURE: [Signature]

DATE: 2/10/92

QUALITY ASSURANCE UNIT AUDIT

AUDITOR: [Signature]

DATE: 2/10/92

SPONSOR:

CIBA-GEIGY Corporation
Agricultural Division
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419

TESTING FACILITY:

CIBA-GEIGY Corporation
Agricultural Division
Method Development Laboratory
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419

PROPOSED EXPERIMENTAL STARTING DATE: February 10, 1992

PROPOSED EXPERIMENTAL COMPLETION DATE: March 20, 1992

PROPOSED STUDY COMPLETION DATE: March 31, 1992
STUDY OBJECTIVES

The objective of this study is to validate "Draft" Analytical Method AG-592¹, shown in Appendix I, for the determination of residues of CGA-152005 in meat, blood, and eggs at a screening level of 0.05 ppm and in milk at a screening level of 0.01 ppm. This validation will be accomplished by analysis of control and fortified control samples to demonstrate the accuracy and precision of the method. Results of the determination of CGA-152005 in meat, milk, blood, and eggs will be reported in Analytical Method AG-592 and in Residue Test Report RI-MV-001-92².

TEST AND REFERENCE SUBSTANCES

CGA-152005: Lot No. S90-1490; B No. 06775; Purity, 97.1%; Reassay Date, 1/93; Storage Condition, room temperature.

A stock standard of CGA-152005 and serial dilutions of the stock standard are stored in the L-2074 refrigerator when not in use. Dilutions of the stock standard will be used for both fortification and quantification.

TEST SYSTEM

Control, fortified control, and ¹⁴C-CGA-152005 containing substrates will be analyzed as described in Table I. The various substrates used in this study are listed below and will be referenced under Test Number RI-MV-001-92.

1. Beef liver, kidney, tenderloin, round, perirenal and omental fat, blood, and milk (Inventory No. 13355.1).

2. Goat liver, omental fat, round, kidney, and milk (Inventory No. 13355.2).

3. Goat liver, omental fat, round, kidney, and milk from Metabolism Study M90-168-001A³ (Inventory No. 13355.4).

4. Poultry liver and peritoneal fat (Inventory No. 13355.5).
(5) Poultry lean meat and eggs from Metabolism Study M91-168-002A4 (Inventory No. 13355.3).

JUSTIFICATION OF TEST SYSTEM

Analysis of control and fortified control beef liver, kidney, tenderloin, round, perirenal and omental fat, blood, and milk as well as poultry liver and peritoneal fat by "Draft" Analytical Method AG-592 (Appendix I) will be performed to determine the accuracy and precision of the method for CGA-152005. Further, analysis of 14C-CGA-152005 containing goat liver, omental fat, round, kidney, and milk5 as well as 14C-CGA-152005 containing poultry lean meat and eggs4 will be performed using AG-592 to determine the extractability and accountability of the method for CGA-152005.

EXPERIMENTAL DESIGN

CIBA-GEIGY "Draft" Analytical Method AG-592 (Appendix I) will be used to determine CGA-152005.

Fortified Samples - "Draft" Analytical Method AG-592 (Appendix I). See also "TEST SYSTEM" Section.

Modifications - Any modifications will be documented with protocol amendments.

The experiments will consist of the analysis of control and fortified control meat, blood, milk, and egg samples, fortified at or above the screening level of "Draft" Analytical Method AG-592 (Appendix I). Several 14C-CGA-152005 containing substrates will also be analyzed.

The sets of samples to be analyzed in this study are outlined in Table I. The accuracy of the method used in this study will be confirmed by the recovery results from the analyses of fortified control samples. The precision of the method will be determined by the reproducibility of the amount of CGA-152005 determined by the method. The accuracy and precision of the method will also be evaluated by
comparing the results of the method (by LC) for the 
$^{14}$C-CGA-152005 containing substrates to the combustion 
analysis results of the same substrates.

The control of bias in the study will be accomplished 
by the use of control samples for all fortification 
experiments. Other experimental design details may be 
found in Appendix I, "Draft" Analytical Method AG-592.

ROUTE OF ADMINISTRATION

Preparation of standards and fortification of control 
samples will be performed according to procedures in 
"Draft" Analytical Method AG-592 (Appendix I). 
Radiolabeled CGA-152005 was administered to goats and 
poultry to provide substrates for metabolism studies 

RECORDS TO BE MAINTAINED

All personnel involved in the study will maintain 
laboratory notebooks or worksheets in which all data 
for the project will be recorded as required by Good 
Laboratory Practice according to the procedures 
outlined in Metabolism and Residue Chemistry SOP 8.1, 
Revision 4. Original chromatograms, computer print-
outs, etc. will be clearly marked as Test Number 
RI-MV-001-92. All data placed in this file will be 
clearly labeled as to origin and referenced to the 
notebook and page of the corresponding work descrip-
tion. Raw data will be archived in the Residue 
Chemistry Archives under Residue Test Report 
RI-MV-001-92$^2$. Results of the method validation will 
be reported in Residue Test Report format. Laboratory 
notebooks will remain in the possession of the analyst 
until the study is completed and then transferred to 
the Residue Department Archives (see Table II).

PROPOSED STATISTICAL METHODS

Statistical methods for regression analysis for a 
standard curve and quantification of residues are 
described in "Draft" Analytical Method AG-592 
(Appendix I).
Recovery results for fortified control samples will be used to calculate accuracy in terms of a mean and standard deviation for the screening level and for all recovery results included in the study.

The precision of the method will also be determined by calculating the mean, range, and standard deviation of triplicate analyses of the C-CGA-152005 containing goat and poultry substrates.

The extractability of the method will be determined by performing liquid scintillation counting measurements of aliquot portions of the extract and comparing those results to the total radioactive residue (TRR) as determined by combustion analysis of an aliquot portion of the same substrate. The accountability of the method will be determined by comparing the results obtained using Method AG-592 to those determined by liquid scintillation counting measurements of aliquot portions of the final fraction used for analysis by liquid chromatography.

PERSONNEL

1. Study Director:
   Robert A. Yokley, Project Scientist.

2. Project Analyst:
   Dan Campbell, Associate Chemist
TABLE I: SAMPLES TO BE ANALYZED

POULTRY LIVER

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Code No.</th>
<th>Fortification Level (ppm)</th>
<th>Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RI-MV-001-92</td>
<td>Reagent Blank</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>RI-MV-001-92</td>
<td>0 (Control)</td>
<td>1</td>
</tr>
<tr>
<td>3, 4</td>
<td>RI-MV-001-92</td>
<td>0.05</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>RI-MV-001-92</td>
<td>0.20</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>RI-MV-001-92</td>
<td>0.50</td>
<td>1</td>
</tr>
</tbody>
</table>

POULTRY PERITONEAL FAT

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Code No.</th>
<th>Fortification Level (ppm)</th>
<th>Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>RI-MV-001-92</td>
<td>Reagent Blank</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>RI-MV-001-92</td>
<td>0 (Control)</td>
<td>1</td>
</tr>
<tr>
<td>9, 10</td>
<td>RI-MV-001-92</td>
<td>0.05</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>RI-MV-001-92</td>
<td>0.20</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>RI-MV-001-92</td>
<td>0.50</td>
<td>1</td>
</tr>
</tbody>
</table>

BEEF LIVER

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Code No.</th>
<th>Fortification Level (ppm)</th>
<th>Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>RI-MV-001-92</td>
<td>0 (Control)</td>
<td>1</td>
</tr>
<tr>
<td>14, 15</td>
<td>RI-MV-001-92</td>
<td>0.05</td>
<td>2</td>
</tr>
<tr>
<td>16</td>
<td>RI-MV-001-92</td>
<td>0.20</td>
<td>1</td>
</tr>
<tr>
<td>17</td>
<td>RI-MV-001-92</td>
<td>0.50</td>
<td>1</td>
</tr>
</tbody>
</table>

BEEF KIDNEY

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Code No.</th>
<th>Fortification Level (ppm)</th>
<th>Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>RI-MV-001-92</td>
<td>0 (Control)</td>
<td>1</td>
</tr>
<tr>
<td>19, 20</td>
<td>RI-MV-001-92</td>
<td>0.05</td>
<td>2</td>
</tr>
<tr>
<td>21</td>
<td>RI-MV-001-92</td>
<td>0.20</td>
<td>1</td>
</tr>
<tr>
<td>22</td>
<td>RI-MV-001-92</td>
<td>0.50</td>
<td>1</td>
</tr>
</tbody>
</table>

BEEF TENDERLOIN

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Code No.</th>
<th>Fortification Level (ppm)</th>
<th>Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>RI-MV-001-92</td>
<td>Reagent Blank</td>
<td>1</td>
</tr>
<tr>
<td>24</td>
<td>RI-MV-001-92</td>
<td>0 (Control)</td>
<td>1</td>
</tr>
<tr>
<td>25, 26</td>
<td>RI-MV-001-92</td>
<td>0.05</td>
<td>2</td>
</tr>
<tr>
<td>27</td>
<td>RI-MV-001-92</td>
<td>0.20</td>
<td>1</td>
</tr>
<tr>
<td>28</td>
<td>RI-MV-001-92</td>
<td>0.50</td>
<td>1</td>
</tr>
</tbody>
</table>
TABLE I: **SAMPLES TO BE ANALYZED**  
(Continued)

**BEEF ROUND**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Code No.</th>
<th>Fortification Level (ppm)</th>
<th>Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>RI-MV-001-92</td>
<td>0 (Control)</td>
<td>1</td>
</tr>
<tr>
<td>30, 31</td>
<td>RI-MV-001-92</td>
<td>0.05</td>
<td>2</td>
</tr>
<tr>
<td>32</td>
<td>RI-MV-001-92</td>
<td>0.20</td>
<td>1</td>
</tr>
<tr>
<td>33</td>
<td>RI-MV-001-92</td>
<td>0.50</td>
<td>1</td>
</tr>
</tbody>
</table>

**BEEF OMENITAL FAT**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Code No.</th>
<th>Fortification Level (ppm)</th>
<th>Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>RI-MV-001-92</td>
<td>0 (Control)</td>
<td>1</td>
</tr>
<tr>
<td>35, 36</td>
<td>RI-MV-001-92</td>
<td>0.05</td>
<td>2</td>
</tr>
<tr>
<td>37</td>
<td>RI-MV-001-92</td>
<td>0.20</td>
<td>1</td>
</tr>
<tr>
<td>38</td>
<td>RI-MV-001-92</td>
<td>0.50</td>
<td>1</td>
</tr>
</tbody>
</table>

**BEEF PERIRENAL FAT**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Code No.</th>
<th>Fortification Level (ppm)</th>
<th>Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>39</td>
<td>RI-MV-001-92</td>
<td>0 (Control)</td>
<td>1</td>
</tr>
<tr>
<td>40, 41</td>
<td>RI-MV-001-92</td>
<td>0.05</td>
<td>2</td>
</tr>
<tr>
<td>42</td>
<td>RI-MV-001-92</td>
<td>0.20</td>
<td>1</td>
</tr>
<tr>
<td>43</td>
<td>RI-MV-001-92</td>
<td>0.50</td>
<td>1</td>
</tr>
</tbody>
</table>

**BEEF BLOOD**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Code No.</th>
<th>Fortification Level (ppm)</th>
<th>Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>44</td>
<td>RI-MV-001-92</td>
<td>Reagent Blank</td>
<td>1</td>
</tr>
<tr>
<td>45</td>
<td>RI-MV-001-92</td>
<td>0 (Control)</td>
<td>1</td>
</tr>
<tr>
<td>46, 47</td>
<td>RI-MV-001-92</td>
<td>0.05</td>
<td>2</td>
</tr>
<tr>
<td>48</td>
<td>RI-MV-001-92</td>
<td>0.20</td>
<td>1</td>
</tr>
<tr>
<td>49</td>
<td>RI-MV-001-92</td>
<td>0.50</td>
<td>1</td>
</tr>
</tbody>
</table>

**MILK**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Code No.</th>
<th>Fortification Level (ppm)</th>
<th>Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>RI-MV-001-92</td>
<td>0 (Control)</td>
<td>1</td>
</tr>
<tr>
<td>51, 52</td>
<td>RI-MV-001-92</td>
<td>0.01</td>
<td>2</td>
</tr>
<tr>
<td>53</td>
<td>RI-MV-001-92</td>
<td>0.10</td>
<td>1</td>
</tr>
<tr>
<td>54</td>
<td>RI-MV-001-92</td>
<td>0.50</td>
<td>1</td>
</tr>
</tbody>
</table>
TABLE I: SAMPLES TO BE ANALYZED
(Continued)

The following sets also contain triplicate analyses of $^{14}$C-CGA-152005 containing substrates.

**POULTRY LEAN MEAT**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Code No.</th>
<th>Fortification Level (ppm)</th>
<th>Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>RI-MV-001-92</td>
<td>0 (Control)</td>
<td>1</td>
</tr>
<tr>
<td>56, 57</td>
<td>RI-MV-001-92</td>
<td>0.05</td>
<td>2</td>
</tr>
<tr>
<td>58</td>
<td>RI-MV-001-92</td>
<td>0.50 (1)</td>
<td>1</td>
</tr>
<tr>
<td>59, 60, 61</td>
<td>H91260507</td>
<td>0.705 (2)</td>
<td>3</td>
</tr>
</tbody>
</table>

**EGGS**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Code No.</th>
<th>Fortification Level (ppm)</th>
<th>Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>62</td>
<td>RI-MV-001-92</td>
<td>0 (Control)</td>
<td>1</td>
</tr>
<tr>
<td>63, 64</td>
<td>RI-MV-001-92</td>
<td>0.05</td>
<td>2</td>
</tr>
<tr>
<td>65</td>
<td>RI-MV-001-92</td>
<td>0.50</td>
<td>1</td>
</tr>
<tr>
<td>66, 67, 68</td>
<td>H91260533</td>
<td>0.326</td>
<td>3</td>
</tr>
</tbody>
</table>

**GOAT LIVER**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Code No.</th>
<th>Fortification Level (ppm)</th>
<th>Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>69</td>
<td>RI-MV-001-92</td>
<td>0 (Control)</td>
<td>1</td>
</tr>
<tr>
<td>70, 71</td>
<td>RI-MV-001-92</td>
<td>0.05</td>
<td>2</td>
</tr>
<tr>
<td>72</td>
<td>RI-MV-001-92</td>
<td>0.10 (1)</td>
<td>1</td>
</tr>
<tr>
<td>73, 74, 75</td>
<td>G90480033</td>
<td>0.058 (3)</td>
<td>3</td>
</tr>
</tbody>
</table>

**GOAT OMENTAL FAT**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Code No.</th>
<th>Fortification Level (ppm)</th>
<th>Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>76</td>
<td>RI-MV-001-92</td>
<td>0 (Control)</td>
<td>1</td>
</tr>
<tr>
<td>77, 78</td>
<td>RI-MV-001-92</td>
<td>0.05</td>
<td>2</td>
</tr>
<tr>
<td>79</td>
<td>RI-MV-001-92</td>
<td>1.50 (1)</td>
<td>1</td>
</tr>
<tr>
<td>80, 81, 82</td>
<td>G90480024</td>
<td>1.335 (3)</td>
<td>3</td>
</tr>
</tbody>
</table>

(1) A fresh fortification near the value of the $^{14}$C-CGA-152005 containing samples.
TABLE I: SAMPLES TO BE ANALYZED (Continued)

GOAT ROUND

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Code No.</th>
<th>Fortification Level (ppm)</th>
<th>Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>83</td>
<td>RI-MV-001-92</td>
<td>0 (Control)</td>
<td>1</td>
</tr>
<tr>
<td>84, 85</td>
<td>RI-MV-001-92</td>
<td>0.05</td>
<td>2</td>
</tr>
<tr>
<td>86</td>
<td>RI-MV-001-92</td>
<td>0.20 (1)</td>
<td>1</td>
</tr>
<tr>
<td>87, 88, 89</td>
<td>G90480031</td>
<td>0.209 (2)</td>
<td>3</td>
</tr>
</tbody>
</table>

GOAT KIDNEY

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Code No.</th>
<th>Fortification Level (ppm)</th>
<th>Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>RI-MV-001-92</td>
<td>0 (Control)</td>
<td>1</td>
</tr>
<tr>
<td>91, 92</td>
<td>RI-MV-001-92</td>
<td>0.05</td>
<td>2</td>
</tr>
<tr>
<td>93</td>
<td>RI-MV-001-92</td>
<td>5.0 (1)</td>
<td>1</td>
</tr>
<tr>
<td>94, 95, 96</td>
<td>G90480026</td>
<td>5.048 (2)</td>
<td>3</td>
</tr>
</tbody>
</table>

GOAT MILK

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Code No.</th>
<th>Fortification Level (ppm)</th>
<th>Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>97</td>
<td>RI-MV-001-92</td>
<td>0 (Control)</td>
<td>1</td>
</tr>
<tr>
<td>98, 99</td>
<td>RI-MV-001-92</td>
<td>0.01</td>
<td>2</td>
</tr>
<tr>
<td>100</td>
<td>RI-MV-001-92</td>
<td>0.10 (1)</td>
<td>1</td>
</tr>
<tr>
<td>101, 102, 103</td>
<td>G90480012</td>
<td>0.069 (2)</td>
<td>3</td>
</tr>
</tbody>
</table>

(1) A fresh fortification near the value of the 14C-GLA-152005 containing samples.
**TABLE II**

**GENERAL INFORMATION**

<table>
<thead>
<tr>
<th>Study Director:</th>
<th>Robert A. Yokley, Project Scientist Method Development, (919) 632-2142</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Analyst:</td>
<td>Dan Campbell, Associate Chemist</td>
</tr>
<tr>
<td>Test Substance:</td>
<td>CGA-152005 for this study (See Test and Reference Substances Section). The metabolism study substrates (goat and poultry) contain $^{14}$C-CGA-152005.</td>
</tr>
<tr>
<td>Testing Facility:</td>
<td>CIBA-GEIGY Corporation Agricultural Division Method Development Laboratory Residue Chemistry Department Post Office Box 18300 410 Swing Road Greensboro, NC 27419</td>
</tr>
<tr>
<td>Archive Location:</td>
<td>The protocol, raw data, final report, and Residue Test Report will be archived in the Residue Chemistry Department Archive at CIBA-GEIGY Corporation, Greensboro, NC. No specimen will remain to be retained.</td>
</tr>
<tr>
<td>Project Number:</td>
<td>168982</td>
</tr>
<tr>
<td>Test Number:</td>
<td>RI-MV-001-92</td>
</tr>
</tbody>
</table>
RADIATION SAFETY COMMITTEE

PROTOCOL FOR VALIDATION OF ANALYTICAL METHOD AG-592

Study/Project No. 168982

Radiolabeled samples from poultry tissues and eggs dosed with $^{14}$C-CGA-152005 according to Metabolism Study M91-168-002A and goat tissues and milk dosed with $^{14}$C-CGA-152005 according to Metabolism Study M90-168-001A are approved for use in this study under Radioactive Materials Project RMP-2.

Signed: W. L. Secrest, Radiation Safety Officer

Date: 2/10/92
REFERENCES:


"DRAFT" ANALYTICAL METHOD FOR THE DETERMINATION
OF CGA-152005 IN MEAT, MILK, BLOOD, AND EGGS BY
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
INCLUDING VALIDATION DATA

ANALYTICAL METHOD NO. 592

CIBA-GEIGY CORPORATION
AGRICULTURAL DIVISION
RESIDUE CHEMISTRY DEPARTMENT
410 SWING ROAD
P. O. BOX 18300
GREENSBORO, NC 27419

PROJECT NUMBER: 168982         PROTOCOL: 26-92

SUBMITTED BY: Daniel D. Campbell

TITLE: Associate Chemist

SIGNATURE:

APPROVED BY: R. K. Williams

TITLE: Manager, Method Development

SIGNATURE:

DATE:

STUDY DIRECTOR: Robert A. Yokley, Ph.D.

SIGNATURE:

COMPLETION DATE:
I. SUMMARY AND INTRODUCTION >
   A. Scope >
   B. Principle >

II. MATERIALS AND METHODS >
   A. Apparatus >
   B. Reagents >
   C. Analytical Procedure >
      1. Extraction >
      2. Partitioning >
      3. Alumina-A Sep-Pak Cleanup >
   D. Instrumentation >
      1. Description and Operating Conditions >
      2. Standardization >
   E. Interferences >
   F. Confirmatory Techniques >
   G. Time Required >
   H. Modifications and Potential Problems >
      1. Emulsion Formation >
      2. Heat >
      3. Degradation in Methanol >
   I. Preparation of Standard Solutions >
      1. Preparation of Analytical Standards >
TABLE OF CONTENTS
(Continued)

PAGE NO.

2. Preparation of Fortification Standards ........................................ >

J. Determination of Sample Residues ...... >

1. Analysis by Linear Regression .... >

2. Calculation of Sample Concentration .................. >

K. Fortification Experiments ................. >

1. Substrate Fortification ................. >

2. Calculation of Procedural Recovery ......................... >

III. RESULTS AND DISCUSSION ......................... >

IV. CONCLUSION ........................................ >

V. CERTIFICATION ......................................... >

VI. QAU STATEMENT ......................................... >

VII. LIST OF TABLES AND FIGURES ...................... >

TABLE I. LIQUID CHROMATOGRAPH OPERATING PARAMETERS FOR THE ANALYSIS OF CGA-152005 ...... >

TABLE II. TYPICAL STANDARDIZATION DATA FOR CGA-152005 ............... >

TABLE III. RECOVERY RESULTS FOR CONTROL AND CGA-152005 FORTIFIED CONTROL SUBSTRATES AND ¹⁴C-CGA-152005 CONTAINING SUBSTRATES ......................... >
TABLE OF CONTENTS
(Continued)

TABLE IV. SUMMARY TABLE FOR THE EXTRACTABILITY, ANALYSIS, AND ACCOUNTABILITY OF 14C-CGA-152005 TREATED SUBSTRATES USING AG-592

FIGURE 1. STRUCTURE AND CHEMICAL NAME OF CGA-152005

FIGURE 2. ANALYTICAL PROCEDURE FLOWCHART FOR AG-592

FIGURE 3. TYPICAL STANDARD CHROMATOGRAMS (FROM THE ANALYSIS OF POULTRY LEAN MEAT)

FIGURE 4. CALIBRATION PLOT OF THE STANDARDS OBTAINED FROM THE ANALYSIS OF POULTRY LEAN MEAT

FIGURE 5. CHROMATOGRAMS OF THE REAGENT BLANKS FROM THE ANALYSIS OF (A) POULTRY LIVER, (B) POULTRY PERITONEAL FAT, (C) BEEF TENDERLOIN, AND (D) BEEF BLOOD

FIGURE 6. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF POULTRY LEAN MEAT FOR CGA-152005 USING METHOD AG-592

FIGURE 7. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF EGGS FOR CGA-152005 USING METHOD AG-592

FIGURE 8. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF GOAT LIVER FOR CGA-152005 USING METHOD AG-592
TABLE OF CONTENTS (Continued)

FIGURE 9. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF GOAT OMENTAL FAT FOR CGA-152005 USING METHOD AG-592 .......... >

FIGURE 10. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF GOAT ROUND FOR CGA-152005 USING METHOD AG-592 ................. >

FIGURE 11. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF GOAT KIDNEY FOR CGA-152005 USING METHOD AG-592 .................. >

FIGURE 12. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF GOAT MILK FOR CGA-152005 USING METHOD AG-592 .................. >

FIGURE 13. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF POULTRY LIVER FOR CGA-152005 USING METHOD AG-592 .................. >

FIGURE 14. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF POULTRY PERITONEAL FAT FOR CGA-152005 USING METHOD AG-592 ............. >

FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF LIVER FOR CGA-152005 USING METHOD AG-592 .................. >

FIGURE 16. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF KIDNEY FOR CGA-152005 USING METHOD AG-592 .................. >
<table>
<thead>
<tr>
<th>PAGE NO.</th>
<th>FIGURE 17.</th>
<th>REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF TENDERLOIN FOR CGA-152005 USING METHOD AG-592 ..........</th>
<th>&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIGURE 18.</td>
<td>REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF ROUND FOR CGA-152005 USING METHOD AG-592 .................</td>
<td>&gt;</td>
<td></td>
</tr>
<tr>
<td>FIGURE 19.</td>
<td>REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF OMental FAT FOR CGA-152005 USING METHOD AG-592 ..........</td>
<td>&gt;</td>
<td></td>
</tr>
<tr>
<td>FIGURE 20.</td>
<td>REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF PERITONEAL FAT FOR CGA-152005 USING METHOD AG-592 ..........</td>
<td>&gt;</td>
<td></td>
</tr>
<tr>
<td>FIGURE 21.</td>
<td>REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF BLOOD FOR CGA-152005 USING METHOD AG-592 ..................</td>
<td>&gt;</td>
<td></td>
</tr>
<tr>
<td>FIGURE 22.</td>
<td>REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF MILK FOR CGA-152005 USING METHOD AG-592 ..................</td>
<td>&gt;</td>
<td></td>
</tr>
</tbody>
</table>

TOTAL NUMBER OF PAGES IS 126
I. SUMMARY AND INTRODUCTION

A. SCOPE

This method is for the determination of residues of CGA-152005 (see figure 1) in animal tissues, milk, blood, and poultry eggs. The limit of detection of this method is 0.06 ng of CGA-152005 and the limit of determination is 0.01 ppm.

B. PRINCIPLE

A 10-g subsample of substrate is homogenized with 90% acetone:10% 0.1% sodium bicarbonate aqueous solution. After filtering, the volume is reduced under vacuum until only the aqueous portion remains. The sample is diluted with 25 ml 0.4% sodium carbonate and partitioned against methyl tert-butyl ether:hexane (1:1). The aqueous phase is acidified with 20 ml 0.8% phosphoric acid, followed by the addition of 20 ml of saturated aqueous salt solution. The compound is extracted by partitioning with dichloromethane:hexane (1:1). The organic portion is dried with sodium sulfate, reduced to about 10 ml and added to a preconditioned alumina-A Sep-Pak column. After column washes with hexane, ethyl acetate, and 1% acetonitrile in ethyl acetate, the compound is eluted with methanol:acetonitrile (60:40). The eluent is concentrated to just dryness. One ml of acetonitrile is added, followed by one ml of picopure water, and the sample is filtered through a 0.45 micron Acrodisc filter. The filter is rinsed with 1 ml of acetonitrile:water (1:1). The sample is analyzed by high performance liquid chromatography (HPLC) using a narrow bore reverse phase column (Supelcosil LC-18-DB) with a mobile phase of acetonitrile:0.05% phosphoric acid in picopure water (40:60). Detection is by UV at 225 nm.
15.0 Syringe, Hamilton Gastight, Luer Tip, l-ml (Fisher #14-824-24 or equivalent)

16.0 Ultrasonicator (Heatsystems Inc. or equivalent)

17.0 Vials, crimp top borosilicate (Sun Broker Inc. or equivalent)

B. REAGENTS

1.0 Acetone, OPTIMA grade (Fisher # A929-4 or equivalent)

2.0 Acetonitrile, HPLC grade (Fisher # A998-4 or equivalent)

3.0 Dichloromethane, HPLC grade (Burdick & Jackson #300-4 or equivalent)

4.0 Ethyl Acetate, HPLC grade (Fisher # E195-4 or equivalent)

5.0 Hexane, HPLC grade (Fisher # H302-4 or equivalent)

6.0 Methanol, HPLC grade (Fisher # A452-4 or equivalent)

7.0 Methyl tert-butyl ether, HPLC grade (Fisher #E127-4)

8.0 Phosphoric acid, Certified ACS grade (Fisher #A242 or equivalent)

9.0 Sodium bicarbonate, Certified ACS grade (Fisher #233-3 or equivalent)

10.0 Sodium carbonate, Certified ACS grade (Fisher #S263-3 or equivalent)

11.0 Sodium chloride, Certified ACS grade (Fisher #5271-3 or equivalent)

12.0 Water, HPLC grade (picopure or equivalent)

C. ANALYTICAL PROCEDURE

1.0 Extraction

1.1 Meat, Blood, Milk, and Eggs

Weigh 10 grams of tissue slices, well mixed blood or egg homogenate, or 50 grams milk into a tared 8-oz. glass bottle. Add 90 ml of 90% acetone:10% 0.1% sodium bicarbonate in picopure water. Homogenize for 30 seconds with the polytron at medium speed. Return any tissues remaining on the polytron blade back to the 8-oz. bottle. Filter through a Reeve Angel #802 filter in a longstem funnel into a 250-ml Erlenmeyer flask. NOTE: The second extraction is not required for the milk samples. Measure and record the volume of the extract for later calculations. Remove the filter paper after all solvent has passed through and place it back into the 8-oz. bottle. Add another 90 ml of 90% acetone:10% 0.1% sodium bicarbonate and homogenize with the polytron for 30 seconds. The generator should be at the bottom of the filter paper and will homogenize the substrate left on the paper, but not the filter itself. A polytron setting slower than the one used in the first extraction may be required. Pour the homogenate through a new Reeve Angel #802 filter and collect with the first extract.
2.0 Partitioning

2.1 Transfer the extract from section 1.1 to a 1000-ml round bottom flask and reduce the solvent by rotary evaporation under vacuum. Lower the flask into a 38 - 40°C warm water bath after about 10 minutes. CAUTION: While bumping does not typically occur in the early stages of the extract reduction, bubbling will occur when most of the organic solvent has been removed. The addition of 10 ml of ethyl acetate to the sample will help if excessive bubbling occurs. Remove the sample when only the aqueous portion remains. Transfer the solution to a 125 ml separatory funnel.

2.2 Add 10 ml of 0.4% sodium carbonate to the 1000-ml round bottom flask, swirl and sonicate to dissolve remaining residues. Transfer to the 125 ml separatory funnel containing the sample. Repeat with another 10 ml of 0.4% sodium carbonate, and end with a third rinse of 5 ml. Add 30 ml of methyl tert-butyl ether (MtBE):hexane (1:1) and shake for one minute, taking care to vent the funnel. Allow the two layers to separate, breaking any emulsions that may form by agitating with a glass pipet or by applying a small amount of heat. Drain the lower aqueous layer and any persistent emulsions into a 250-ml Erlenmeyer flask and discard the organic layer. Pour the aqueous phase back into the 125-ml separatory funnel and repeat the extraction with 30 ml of
MtBE:hexane (1:1). Collect the aqueous layer into the same Erlenmeyer flask.

2.3 Add 20 ml of 0.8% H₃PO₄ (in picopure water) and 20 ml of picopure water saturated with sodium chloride to the aqueous phase in the Erlenmeyer flask from section C.2.2. Transfer to a 250-ml separatory funnel and extract 3 times by partitioning with 60 ml dichloromethane:hexane (1:1), taking care to vent the funnel. The first 60 ml of dichloromethane:hexane (1:1) used for the partitioning should also be used to rinse the 250-ml Erlenmeyer flask. Treat emulsions that form as was done in section C.2.2, and combine and save the upper organic layers in a separate 500-ml Erlenmeyer flask.

3.0 Alumina-A Sep-Pak Cleanup

3.1 Attach a 50-ml Analytichem reservoir to an Alumina-A Sep-Pak column, and condition the column with the following rinses: 25 ml methanol:acetonitrile (60:40), 15 ml ethyl acetate, and 25 ml dichloromethane (DCM):hexane (1:1). Do not let the column dry between rinses and prior to applying the sample.

3.2 Add approximately 15 grams of sodium sulfate directly to the organic phase from section C.2.3 to bind any water in the extract, and transfer to a 500 ml round bottom flask. Rinse the sodium sulfate 3 times with small volumes (~3-5 ml) of DCM:hexane (1:1). Reduce the volume of the sample under
vacuum by rotary evaporation in a 35°C warm water bath to 5 to 10 ml, taking care not to let the sample go to dryness.

3.3 Add the sample from section C.3.2 to the conditioned Alumina-A Sep-Pak column. Rinse the round bottom flask 3 times with 1 to 2 ml of DCM:hexane (1:1), pouring each rinse into the reservoir above the Alumina-A column. Allow the sample and washes to drip through the column by gravity only. The flow may occasionally stop and can be restarted by applying gentle pressure to the top of the reservoir with a pipet bulb. Wash with the following, being careful to insure that the column does not go dry between rinses. Collect the eluent in a 100-ml round bottom flask.

Wash with 15 ml hexane (discard)
Wash with 25 ml ethyl acetate (discard)
Wash with 15 ml 1½ acetonitrile in ethyl acetate (discard)
Elute with 30 ml 60:40 methanol: acetonitrile (collect)

3.4 Reduce the eluant volume from section 3.3 to just dryness under vacuum by rotary evaporation in a 35°C water bath. This step must be done soon after elution from the Alumina-A column since preliminary reports indicate that CGA-152005 is unstable in methanol for prolonged periods.
of time. When the sample has just reached dryness, remove it from the rotary evaporator and add 1 ml acetonitrile. Swirl and sonicate to dissolve all residues. Add 1 ml picopure water and swirl and sonicate a second time. For milk samples at 0.01 ppm, increase the volumes to 3 ml acetonitrile and 3 ml water.

3.5 Prewash a 0.45 μm, 25 mm Acrodisc PVDF HPLC syringe filter with 3 X 1ml acetonitrile: picopure water (1:1). Filter the sample from section 3.4 slowly through the Acrodisc using a 1-ml glass syringe. Add 1 ml acetonitrile: picopure water (1:1) to the round bottom flask, swirl and pass through the same filter as a rinse. For 0.01 ppm milk samples, increase the volume of the rinse to 4 ml for a total of 10 ml in the final sample. Mix the sample well and transfer 1 to 2 ml to a borosilicate crimp-top vial for analysis by HPLC.

D. INSTRUMENTATION

1.0 Description and Operating Conditions

Residues of CGA-152005 are determined by HPLC on a narrow bore base deactivated Supelco C-18 column (LC-18-DB). The mobile phase is 60% 0.05% phosphoric acid in picopure water: 40% acetonitrile at a flow rate of 0.3 ml per minute. Detection of CGA-152005 is
performed using an ultraviolet
detector at a wavelength of 225 nm.
The injection volume is 10 μl. The
HPLC conditions are listed in
Table 1.

2.0 Standardization

Standardize the HPLC system by
injecting 10-μl aliquots of
standard solutions of CGA-152005 in
a working range of 0.6-10
ng/injection. Generate a linear
regression from the data by
comparing detector response and ng
injected. See section I.2.0 for
preparation of analytical
standards.

E. INTERFERENCES

None.

F. CONFIRMATORY TECHNIQUES

None.

G. TIME REQUIRED

The extraction and cleanup of a set of 6
samples may be completed within a time
period of 8 hours. HPLC analysis can be
performed overnight using automatic
injection.

H. MODIFICATIONS AND POTENTIAL PROBLEMS

1.0 Emulsion Formation

Some samples may develop emulsions
during partitioning (during steps
in sections C.2.1 and C.2.2).
These may be cleared if allowed to
settle out and then gently stirred
with a glass rod. Slight heating
may help (a hair dryer was used),
but care must be taken to avoid
temperatures in excess of 40°C. Any
emulsions that remain should stay
with the aqueous phases during the
partitioning.
2.0 Heat

During the evaporation of the solvents from the samples in sections C.2.1, C.3.2, and C.3.4, any water bath used must not have a temperature >40°C and the samples should be removed just as soon as they reach dryness. A temperature of 35°C is required for removal of the solvent from the Alumina-A eluent. Excessive temperature, especially when the sample has gone to dryness, may lead to analyte decomposition.

3.0 Degradation in Methanol

After elution from the Alumina-A column, the solvent must be evaporated as soon as possible, since CGA-152005 was reported to be unstable in methanol. Do not store the sample overnight in the methanol:acetonitrile eluent.

I. PREPARATION OF STANDARD SOLUTIONS

1.0 Preparation of Analytical Standards

Weigh 10 mg of CGA-152005 analytical standard into a 100-ml volumetric flask and dilute to the mark with acetonitrile. Make serial dilutions of the 0.1 mg/ml standard solution with 50% acetonitrile: 50% picpure water to give a series of fortification/analytical standards in a range of 0.06 µg/ml to 1.0 µg/ml of CGA-152005. Store the standard solutions in amber bottles at 4°C in the dark when not in use.

2.0 Preparation of Analytical Standards

The 1.0 µg/ml standard used to fortify standards is prepared in section I.1.0 above. See section K.1.0 for fortification procedures.
J. DETERMINATION OF SAMPLE RESIDUES

1.0 Linear Regression Analysis

Inject 10-ul aliquots of sample extracts onto the HPLC system. Compare the analyte peak height found in the sample extracts to the peak heights determined for the analytical standards by entering them into a linear regression program or by using a computer system (e.g. VG Multichrom) to determine the nanograms of CGA-152005 in the injected aliquot.

2.0 Calculation of Concentration

Calculate the residue results in terms of ppm of CGA-152005 by using the following equation:

\[
(1) \quad \text{ppm} = \frac{\text{(ng CGA-152005 Found)}}{\text{(mg sample injected)}}
\]

Where the ng CGA-152005 is obtained from the linear regression analysis in section J.1.0 and the mg sample injected is calculated as follows: (Equation 2)

\[
(2) \quad \text{mg inj.} = \frac{G}{(V_a)} \left( \frac{V_i}{V_f} \right)
\]

G = milligrams sample extracted

\( V_a \) = aliquot volume (from section C.1.1)

Ve = extraction volume (from section C.1.1; usually 90 ml for milk and 180 ml for other substrates)

\( V_i \) = injection volume (ul)

\( V_f \) = total volume of final injection solution (ul)
K. Fortification Experiments

1.0 Substrate Fortification

This method is validated for each set of samples analyzed by including an untreated control sample and one or more control samples fortified immediately prior to extraction with CGA-152005. Add 0.5 ml of a 1.0 ug/ml standard solution of CGA-152005 to 10 g. of control tissues or 50 g of milk for a 0.05 ppm or 0.01 ppm fortification, respectively. Allow the solvent to evaporate for at least 20 minutes prior to addition of extraction solvent. Analyze control and freshly fortified samples along with the treated samples according to the procedures of the method.

2.0 Calculation of Procedural Recovery

The final ppm value of the control and fortified samples can be obtained using the calculations shown in section J.2.0. Determine the % recovery by first subtracting the background detector response, if any, in the control sample from the CGA-152005 response in the recovery sample. Calculate the recovery factor as a percentage (R) by the equation:

\[
R\% = \frac{\text{ppm CGA-152005 found}}{\text{ppm CGA-152005 added}} \times 100
\]

III. RESULTS AND DISCUSSION

IV. CONCLUSION
V. CERTIFICATION

The reports and experimental results included in this study, Laboratory Project I.D. AG-592, are certified to be authentic accounts of the experiments.

Robert K. Williams, Manager  
Method Development  
Residue Chemistry Department  
919-632-2295  

Date

CERTIFICATION OF GOOD LABORATORY PRACTICES

The analytical work reported in AG-592 was performed in accordance with Good Laboratory Practice Standards, 40 CFR Part 160.

Robert A. Yokley, Ph.D  
Study Director  

Date
VI. OAU STATEMENT
TABLE I. LIQUID CHROMATOGRAPHIC OPERATING PARAMETERS FOR THE ANALYSIS OF CGA-152005

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument</td>
<td>Perkin-Elmer Model Series-4 Solvent Delivery System or equivalent. Perkin-Elmer Model ISS-100 Automatic HPLC sampler or equivalent.</td>
</tr>
<tr>
<td>Column Oven</td>
<td>BioRad HPLC column heater, model number 125-0425</td>
</tr>
<tr>
<td>Oven Temp.</td>
<td>30°C</td>
</tr>
<tr>
<td>Column</td>
<td>Supelcosil LC-18-DB, 250mm x 2.1 mm, 5 um particle size (Supelco cat. #5-7940M) with Supelguard LC-18-DB guard column.</td>
</tr>
<tr>
<td>Mobile Phase</td>
<td>60% 0.05% phosphoric acid in picopure water:40% acetonitrile</td>
</tr>
<tr>
<td>Retention Time</td>
<td>~22 minutes</td>
</tr>
<tr>
<td>Detection</td>
<td>ABI Kratos Spectroflow Model 783 Programmable Absorbance Detector or equivalent variable wavelength detector.</td>
</tr>
<tr>
<td>Wavelength</td>
<td>225 nm</td>
</tr>
<tr>
<td>Attenuation</td>
<td>0.006 AUFS</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>0.3 mL/min</td>
</tr>
<tr>
<td>Volume Injected</td>
<td>10 ul</td>
</tr>
<tr>
<td>Chart Speed</td>
<td>0.25 cm/min</td>
</tr>
<tr>
<td>Run Time</td>
<td>30 min/injection</td>
</tr>
<tr>
<td>Data Acquisition</td>
<td>Microvax II (Bones) Operating System, VMS Version 5.3-1 Application Software: VG Multichrom Version 1.8 Worksheet Version: Ws.pas 1.3.1</td>
</tr>
</tbody>
</table>
TABLE II.  TYPICAL STANDARDIZATION DATA FOR CGA-152005
TABLE III. RECOVERY RESULTS FOR CONTROL AND CGA-152005 FORTIFIED CONTROL SUBSTRATES AND 14C-CGA-152005 CONTAINING SUBSTRATES
TABLE IV. SUMMARY TABLE FOR EXTRACTABILITY ANALYSIS AND ACCOUNTABILITY OF \(^{14}\text{C-CGA-152005 TREATED SUBSTRATE USING AG-592}\)
FIGURE 1. STRUCTURE AND CHEMICAL NAME OF CGA-152005

CGA-152005

1-(4-Methoxy-6-methyl-triazin-2-yl)-3-[2-(3,3,3-trifluoropropyl)-phenylsulfonyl]-urea
FIGURE 2. ANALYTICAL PROCEDURE FLOWCHART FOR AG-592

10 g. sample for tissues, blood or egg. 50 g. sample for milk.

Homogenize with 90 ml 90\% acetone:10\% 0.1\% sodium bicarbonate, 30 seconds.

Filter (R&A #802 filter)

Place filter with residual tissues into bottle and homogenize again with 90 ml 90\% acetone:10\% 0.1\% sodium bicarbonate. Refilter (R&A #802) and combine extracts.

Measure volume and remove acetone via rotary evaporation (38-40°C)

Add 25 ml 0.4\% sodium carbonate. Partition (2 x 30 ml) with methyl tert-butyl ether:hexane (1:1). Discard organic.

(aqueous)

Add 20 ml 0.8\% phosphoric acid and 20 ml water saturated with sodium chloride in water. Partition 3 x 60 ml with dichloromethane:hexane (1:1). Discard aqueous.

(organic)

Dry with sodium sulfate and reduce to 10 ml via rotary evaporation (35°C).

↓ Alumina A Sep-Pak Solid Phase Extraction

Condition column with 25 ml methanol:acetonitrile (1:1), 25 ml ethyl acetate, and 25 ml dichloromethane:hexane (1:1)

Add sample (quantitatively)

Wash column with 15 ml hexane (discard wash)

Wash column with 25 ml ethyl acetate (discard wash)

Wash column with 1\% acetonitrile in ethyl acetate (discard wash)

Elute sample with 30 ml methanol:acetonitrile (60:40). Collect in 100-ml round bottom flask.

↓ Evaporate to just dryness with rotovap (35°C)

Add 1 ml acetonitrile. Sonicate and swirl.

Add 1 ml picopure water. Sonicate and swirl. Filter through Acrodisc LC PVDF 25 mm diameter, 0.45 um pore filter.

Rinse round bottom and filter with 1 ml acetonitrile:water (1:1)

↓ HPLC

(Supelcosil LC-CB-18, 25 cm x 2.1 mm; mobile phase = 60\% 0.05\% phosphoric acid in picopure water:40\% acetonitrile; UV detection at 225 nm)
FIGURE 3. TYPICAL STANDARD CHROMATOGRAMS (FROM THE ANALYSIS OF POULTRY LEAN MEAT)
FIGURE 4. CALIBRATION PLOT OF THE STANDARDS OBTAINED FROM THE ANALYSIS OF POULTRY LEAN MEAT
FIGURE 5. CHROMATOGRAMS OF REAGENT BLANKS FROM THE ANALYSIS OF (A) POULTRY LIVER, (B) POULTRY PERIONEAL FAT, (C) BEEF TENDERLOIN, AND (D) BEEF BLOOD.
FIGURE 6. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF POULTRY LEAN MEAT FOR CGA-152005 USING METHOD AG-592
FIGURE 7. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF EGGS FOR CGA-152005 USING METHOD AG-592
FIGURE 8. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF GOAT LIVER FOR CGA-152005 USING METHOD AG-592
FIGURE 9. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF GOAT OMENTAL FAT FOR CGA-152005 USING METHOD AG-592
FIGURE 10. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF GOAT ROUND FOR CGA-152005 USING METHOD AG-592
FIGURE 11. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF GOAT KIDNEY FOR CGA-152005 USING METHOD AG-592
FIGURE 12. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF GOAT MILK FOR CGA-152005 USING METHOD AG-592
FIGURE 14. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF POULTRY PERITONEAL FAT FOR CGA-152005 USING METHOD AG-592
FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF LIVER FOR CGA-152005 USING METHOD AG-592
FIGURE 17. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF TENDERLOIN FOR CGA-152005 USING METHOD AG-592
FIGURE 18. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF ROUND FOR CGA-152005 USING METHOD AG-592
FIGURE 19. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF OMENAL FAT FOR CGA-152005 USING METHOD AG-592
FIGURE 20. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF EGG AND BEEF PERITONEAL FAT CGA-152005 USING METHOD AG-592
FIGURE 21. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF BEEF BLOOD FOR CGA-152005 USING METHOD AG-592
RESIDUE CHEMISTRY PROTOCOL AMENDMENT

AMENDMENT NUMBER: 1

PROTOCOL NUMBER: 26-92


PROJECT NUMBER (S): 168982

CHANGE(S):
(1) The following statement should be added to Protocol 26-92 on page 5 after the Proposed Statistical Methods Section.

MODIFICATIONS — Any modifications which alter the chemical procedure, solvent ratios, sample size, or sample identity will be documented with protocol amendments. It is expected that editorial alterations and changes in format will be made to the "Draft" Analytical Method before it is issued. These changes will not require amendment documentation since they will be documented in the validated Analytical Method which will be approved as a final report.

(2) The 14C-CGA-152005 concentrations listed in Table I on page 8 are listed as 0.058 ppm for goat liver and 1.335 ppm for goat omental fat. These are incorrect. The correct values are 1.371 ppm for goat liver and 0.058 ppm for goat omental fat.

(3) A footnote should be added for the egg samples listed in Table I on page 8. The fresh fortifications are on whole eggs whereas only the yolks were used for the 14C-CGA-152005 containing samples.

(4) The sample size is listed as 10 grams in the method. However, the sample size for the 14C-CGA-152005 containing egg yolk samples listed in Table I on page 8 was 5 grams during the method validation.

(5) A Submitter/Sponsor signature and date section will be added to the certification page.

(6) In Section C.3.3 of AG-592, the third wash of the alumina A column is listed as 1% ACN in ethyl acetate. This is incorrect. The correct percentage is 5% ACN in ethyl acetate.
(7) A statement was added to Section C.3.5 to address the final dilution volumes for samples containing high concentrations of CGA-152005 so that the samples are sufficiently diluted to insure peak heights within the calibration range of the injected standards.

(8) The final dilution volume for the 0.010 ppm fortification level milk samples was listed as 4 ml on page 14. This should be listed as 3 ml.

(9) It was listed in the protocol that the goat milk fortification (Table I, page 9) would be 0.010 ppm for the low fortification level. The actual fortification during the method validation was 0.050 ppm for the low fortification level.

REASON(S):

(1) This statement will allow editorial changes to be made to the method without requiring a protocol amendment for every minor change that does not affect the quality and/or integrity of the study.

(2) We reported the numbers supplied to us by metabolism personnel in the protocol. We realized the error when we analyzed the 14C-CGA-152005 containing goat liver and the peaks were much larger than expected. A review of the combustion data revealed that we had been supplied the wrong numbers for goat liver and goat omental fat. The goat liver analyses had to be repeated.

(3) The yolks were available and contained sufficient activity for the method validation.

(4) Only about 18 grams were available so the sample size had to be reduced to perform triplicate analyses.

(5) The absence of this submitter/sponsor signature and date section was cited in an In-Progress audit of another study. It has been added here to maintain GLP compliance.

(6) This was a proof reading oversight. The wash actually used was the 5% ACN in ethyl acetate solution.

(7) This was inadvertently omitted from the method and should be included.

(8) An oversight. The correct volume is 3 ml.

(9) We intended to fortify goat milk at the 0.010 ppm level but the samples were inadvertently fortified at the 0.050 ppm level instead. However, This does not adversely affect the results of this study since the radiolabelled samples contained CGA-152005 concentrations greater than 0.05 ppm. In
addition, the results of the analysis of dairy milk demonstrated the ability of the method to screen at the 0.010 ppm level.

EFFECTIVE DATE(S): March 20, 1992

CHANGE INITIATED BY: Robert A. Yoffey
CURRENT STUDY DIRECTOR

STUDY ACCEPTED BY: ________________________________ DATE: ___________

(If Applicable) NEW STUDY DIRECTOR

CHANGE AUTHORIZED BY: ________________________________ DATE: ___________

(If Applicable) MANAGEMENT SIGNATURE

CONTRACT LABORATORY STUDY COORDINATOR: ________________________________ DATE: ___________

(If Applicable)

AMENDMENTS TO BE DISTRIBUTED PER PROTOCOL DISTRIBUTION LIST

PAGE 1 OF 3
APPENDIX II

SEPARATE DOCUMENTS ACCOMPANYING THIS REPORT


SUBMITTER/SPONSOR:
Ciba Plant Protection
Ciba-Geigy Corporation
Post Office Box 18300
Greensboro, NC 27410
APPENDIX IV

RESIDUE TEST REPORT RI-MV-001-92 REPORT NO. 1

SUBMITTER/SPONSOR:
Ciba Plant Protection
Ciba-Geigy Corporation
Post Office Box 10300
Greensboro, NC 27410
RESIDUE CHEMISTRY DEPARTMENT
AGRICULTURAL DIVISION
CIBA-GEIGY CORPORATION
GREENSBORO, NORTH CAROLINA

RESIDUE TEST REPORT

FIELD TEST NUMBER: RI-MV-001-92
REPORT NO.: 01

PROJECT NUMBER: 168982
PROTOCOL NUMBER: 26-92 and Amendment #1
TEST SUBSTANCE: CGA-152005
TEST SYSTEM: Poultry, Goat, and Beef Tissues, Milk, and Blood
LOCATION: Not Applicable
AG: 592 NO. OF ANALYSES: 99
LABORATORY: CIBA-GEIGY Method Development

DESCRIPTION: "Draft" Method AG-592 was validated for the determination of CGA-152005 by analyzing control samples, fortified control samples, and samples treated/dosed with 14C-CGA-152005 which were obtained from metabolism studies. Radioactive extractability and accountability determinations were also performed on the incurred 14C-CGA-152005 residues in treated poultry and goat substrates.

STUDY DIRECTOR: Robert A. Yokley, Ph.D.
Project Scientist

SIGNATURE: 

DATE: 3-31-92

APPROVED BY: R. K. Williams
Manager, Method Development

SIGNATURE: 

APPROVAL DATE: 3/31/92

DISTRIBUTION: D. D. Campbell
R. A. Kahrs
R. K. Williams
R. A. Yokley
Main File
RESIDUE TEST REPORT

FIELD TEST NUMBER: RI-MV-001-92
REPORT NUMBER: 01
PROTOCOL NUMBER: 26-92
PROJECT NUMBER: 168982

BIOLOGY SECTION

The various substrates used in this study are referenced under Test Number RI-MV-001-92 and include the below listed substrates.

(1) Beef liver, kidney, tenderloin, round, perirenal and omental fat, blood, and milk (Inventory Number 13355.1).

(2) Goat liver, omental fat, round, kidney, and milk (Inventory Number 13355.2).

(3) Poultry liver and peritoneal fat (Inventory Number 13355.5).

(4) Goat liver, omental fat, round, kidney, and milk from Metabolism Study M90-168-001A, Protocol Number 169-90, In-Progress (Inventory Number 13355.4).

(5) Poultry lean meat and eggs from Metabolism Study M91-168-002A, Protocol Number 196-90, In-Progress (Inventory Number 13355.3).

CIRCUMSTANCES AFFECTING THE QUALITY OR INTEGRITY OF THE DATA

None

SAMPLE IDENTIFICATION NUMBERS

Each sample was assigned a specific sample code number in Protocol 26-92. This sample code was used in laboratory notebook 4235.

STUDY PERSONNEL

D. D. Campbell, Associate Chemist (DDC)
R. A. Yokley, Project Scientist (RAY)
RESIDUE TEST REPORT

FIELD TEST NUMBER: RI-MV-001-92
REPORT NUMBER: 01

PROTOCOL NUMBER: 26-92
PROJECT NUMBER: 168982

ANALYTICAL SECTION

METHODOLOGY

METHOD NUMBER
AG-592

COMMENTS
All data were acquired using a VG Multichrom Version 1.8 or 2.0 data acquisition system. Liquid scintillation counting was performed according to SOP 4.6, Revision Number 2 and the data were calculated manually. An example calculation is shown in AG-592.

TEST SUBSTANCES AND CALIBRATION STANDARDS

ANALYTICAL STANDARD
CGA-152005

LOT NUMBER
S90-1490 (806775)

PURITY
97.1%

REANALYSIS DATE
1/93

SUMMARY
The validity of "Draft" Analytical Method AG-592 for the determination of CGA-152005 was tested by analyzing control samples, fortified control samples, and samples of animal tissues, milk, and blood treated/dosed with 14C-CGA-152005 from metabolism studies M90-168-001A and M91-168-001A (in-progress) in order to determine the extractability, accountability, precision, and accuracy of the method.

CONCLUSION
Method AG-592 is a valid method for the determination of CGA-152005 in meat, milk, eggs, and blood.
RESIDUE TEST REPORT

FIELD TEST NUMBER: RI-MV-001-92
REPORT NUMBER: 01

PROTOCOL NUMBER: 26-92
PROJECT NUMBER: 168982

ANALYTICAL SECTION

RESULTS

The accuracy of the method is demonstrated by the results shown in Table I in terms of fresh fortification level, quantity found, and % recovery. The mean for each set of recoveries ranged from 75% to 104%. The average recovery for all substrates was 90% with a standard deviation of 11%.

The precision of the method is demonstrated by the results shown in Table II in which the ppm found from triplicate analyses of the $^{14}$C-CGA-152005 containing substrates are tabulated. The standard deviations range from 0.002% for goat omental fat (mean = 0.082 ppm) to 0.053% for goat kidney (mean = 5.4 ppm).

Table III shows the extractability and accountability data. The extractability was calculated by comparing (by percentages) the concentration of the total radioactive residue found in the extract, as determined by liquid scintillation counting, with the total radioactive residue in the sample, as preliminarily reported in metabolism studies M90-168-001A and M91-168-002A (In-progress). The extractabilities ranged from 72% for eggs to 148% for goat omental fat.

The % accountability was calculated by comparing (by percentages) the concentration of CGA-152005 in the final fractions, as determined by liquid chromatography, with the $^{14}$C-CGA-152005 concentration in the sample, as reported in metabolism studies M90-168-001A and M91-168-002A (In-progress). The accountabilities ranged from 28% for eggs to 142% for goat omental fat.

The limit of detection is 0.60 ng CGA-152005 and the limit of determination (screening level) is 0.05 ppm CGA-152005 for all substrates except milk which has a screening level of 0.01 ppm.
### TABLE I.

**RECOVERY RESULTS FOR CONTROL AND CGA-152005 FORTIFIED CONTROL SUBSTRATES USING METHOD AG-592**

<table>
<thead>
<tr>
<th>(1) <strong>SUBSTRATE</strong></th>
<th>PROTOCOL 26-92 SAMPLE #</th>
<th>CGA-152005 FORTIFICATION (ppm)</th>
<th>CGA-152005 (ppm)</th>
<th>(2) % RECOVERY (AG-592)</th>
<th>% RECOVERY STANDARD DEVIATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poultry</td>
<td>02</td>
<td>0</td>
<td>&lt;0.05</td>
<td>103</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>03</td>
<td>0.05</td>
<td>0.051</td>
<td>96</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>04</td>
<td>0.05</td>
<td>0.048</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>05</td>
<td>0.20</td>
<td>0.20</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td></td>
<td>06</td>
<td>0.50</td>
<td>0.48</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>Poultry</td>
<td>08</td>
<td>0</td>
<td>&lt;0.05</td>
<td>89</td>
<td>88</td>
</tr>
<tr>
<td>Pancreas</td>
<td>09</td>
<td>0.05</td>
<td>0.044</td>
<td>91</td>
<td>3.1</td>
</tr>
<tr>
<td>Fat</td>
<td>10</td>
<td>0.05</td>
<td>0.045</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>0.20</td>
<td>0.18</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.50</td>
<td>0.42</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>Beef</td>
<td>13</td>
<td>0</td>
<td>&lt;0.05</td>
<td>106</td>
<td>104</td>
</tr>
<tr>
<td>Liver</td>
<td>14</td>
<td>0.05</td>
<td>0.053</td>
<td>101</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0.05</td>
<td>0.055</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>0.20</td>
<td>0.20</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>0.50</td>
<td>0.49</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>Beef</td>
<td>18</td>
<td>0</td>
<td>&lt;0.05</td>
<td>83</td>
<td>91</td>
</tr>
<tr>
<td>Kidney</td>
<td>19</td>
<td>0.05</td>
<td>0.047</td>
<td>92</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.05</td>
<td>0.050</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>0.20</td>
<td>0.19</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>0.50</td>
<td>0.50</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>Beef</td>
<td>24</td>
<td>0</td>
<td>&lt;0.05</td>
<td>93</td>
<td>98</td>
</tr>
<tr>
<td>Tenderloin</td>
<td>25</td>
<td>0.05</td>
<td>0.047</td>
<td>94</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>0.05</td>
<td>0.047</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>0.20</td>
<td>0.22</td>
<td>108</td>
<td></td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>0.50</td>
<td>0.48</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>Beef</td>
<td>29</td>
<td>0</td>
<td>&lt;0.05</td>
<td>91</td>
<td>79</td>
</tr>
<tr>
<td>Round</td>
<td>30</td>
<td>0.05</td>
<td>0.046</td>
<td>84</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>0.05</td>
<td>0.042</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>0.20</td>
<td>0.139</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>0.50</td>
<td>0.363</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>Beef</td>
<td>34</td>
<td>0</td>
<td>&lt;0.05</td>
<td>82</td>
<td>82</td>
</tr>
<tr>
<td>Omental Fat</td>
<td>35</td>
<td>0.05</td>
<td>0.041</td>
<td>82</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>0.05</td>
<td>0.050</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>0.20</td>
<td>0.16</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>0.50</td>
<td>0.46</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>Beef</td>
<td>39</td>
<td>0</td>
<td>&lt;0.05</td>
<td>79</td>
<td>87</td>
</tr>
<tr>
<td>Penneal Fat</td>
<td>40</td>
<td>0.05</td>
<td>0.040</td>
<td>79</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>41</td>
<td>0.05</td>
<td>0.047</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>0.20</td>
<td>0.17</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>0.50</td>
<td>0.45</td>
<td>90</td>
<td></td>
</tr>
</tbody>
</table>

(1) All samples are from Test Number RJ-MV-001-92 - overall mean of 90% with a SD of 11% (n=61).
(2) Recovery samples were corrected for control residues where present.
## TABLE I.

**RECOVERY RESULTS FOR CONTROL AND CGA-152005 FORTIFIED CONTROL SUBSTRATES USING METHOD AG-592**

*(Continued)*

<table>
<thead>
<tr>
<th>(1) SUBSTRATE</th>
<th>(2) CGA-152005 FORTIFICATION (ppm)</th>
<th>(2) CGA-152005 % RECOVERY (AG-592)</th>
<th>% RECOVERY MEAN</th>
<th>STANDARD DEVIATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef Blood</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>0</td>
<td>&lt;0.05</td>
<td>78</td>
<td>16</td>
</tr>
<tr>
<td>46</td>
<td>0.05</td>
<td>0.046</td>
<td></td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>0.05</td>
<td>0.044</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>0.20</td>
<td>0.15</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>0.50</td>
<td>0.29</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Beef Milk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>0</td>
<td>&lt;0.01</td>
<td>86</td>
<td>8.2</td>
</tr>
<tr>
<td>51</td>
<td>0.01</td>
<td>0.0082</td>
<td></td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>0.01</td>
<td>0.010</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>53</td>
<td>0.10</td>
<td>0.084</td>
<td>97</td>
<td>5.1</td>
</tr>
<tr>
<td>54</td>
<td>0.50</td>
<td>0.393</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poultry</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>0</td>
<td>&lt;0.05</td>
<td>87</td>
<td>2.3</td>
</tr>
<tr>
<td>56</td>
<td>0.05</td>
<td>0.049</td>
<td></td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>0.05</td>
<td>0.051</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>0.50</td>
<td>0.46</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>Lean Meat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>62</td>
<td>0</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>63</td>
<td>0.05</td>
<td>0.045</td>
<td></td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>0.05</td>
<td>0.042</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>0.50</td>
<td>0.43</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>Eggs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>0</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>0.05</td>
<td>0.054</td>
<td></td>
<td></td>
</tr>
<tr>
<td>71</td>
<td>0.05</td>
<td>0.051</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>1.00</td>
<td>1.02</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>Goat Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>76</td>
<td>0</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>77</td>
<td>0.05</td>
<td>0.036</td>
<td></td>
<td></td>
</tr>
<tr>
<td>78</td>
<td>0.05</td>
<td>0.037</td>
<td></td>
<td></td>
</tr>
<tr>
<td>79</td>
<td>0.10</td>
<td>0.079</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>83</td>
<td>0</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>84</td>
<td>0.05</td>
<td>0.051</td>
<td></td>
<td></td>
</tr>
<tr>
<td>85</td>
<td>0.05</td>
<td>0.051</td>
<td></td>
<td></td>
</tr>
<tr>
<td>86</td>
<td>0.20</td>
<td>0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omental Fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>87</td>
<td>0</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>88</td>
<td>0.05</td>
<td>0.051</td>
<td></td>
<td></td>
</tr>
<tr>
<td>89</td>
<td>0.10</td>
<td>0.079</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goat Round</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>0</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>91</td>
<td>0.05</td>
<td>0.050</td>
<td></td>
<td></td>
</tr>
<tr>
<td>92</td>
<td>0.05</td>
<td>0.053</td>
<td></td>
<td></td>
</tr>
<tr>
<td>93</td>
<td>0.05</td>
<td>0.053</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goat Kidney</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>97</td>
<td>0</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>98</td>
<td>0.05</td>
<td>0.043</td>
<td></td>
<td></td>
</tr>
<tr>
<td>99</td>
<td>0.20</td>
<td>0.093</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0.10</td>
<td>0.071</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(1) All samples are from Test Number RI-MV-001-92 - overall mean of 90% with a SD of 11% (n=61).

(2) Recovery samples were corrected for control residues where present.
TABLE II. PRECISION OF ANALYTICAL METHOD AG-592 AS DEMONSTRATED BY THE RESULTS OF ANALYZING $^{14}$C-CGA-152005 CONTAINING SUBSTRATES

<table>
<thead>
<tr>
<th>SUBSTRATE</th>
<th>PROTOCOL 25-92 SAMPLE #</th>
<th>(1) FOUND BY AG-592 (ppm)</th>
<th>MEAN (ppm)</th>
<th>RANGE (ppm)</th>
<th>STANDARD DEVIATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poultry Lean Meat</td>
<td>59, 60, 61</td>
<td>0.64, 0.62, 0.65</td>
<td>0.63</td>
<td>0.62-0.65</td>
<td>0.015</td>
</tr>
<tr>
<td>Eggs</td>
<td>66, 67, 68</td>
<td>0.099, 0.087, 0.088</td>
<td>0.091</td>
<td>0.087-0.099</td>
<td>0.006</td>
</tr>
<tr>
<td>Goat Liver</td>
<td>73, 74, 75</td>
<td>1.37, 1.44, 1.42</td>
<td>1.41</td>
<td>1.37-1.44</td>
<td>0.037</td>
</tr>
<tr>
<td>Goat Omental Fat</td>
<td>80, 81, 82</td>
<td>0.082, 0.081, 0.084</td>
<td>0.082</td>
<td>0.081-0.084</td>
<td>0.002</td>
</tr>
<tr>
<td>Goat Round</td>
<td>87, 88, 89</td>
<td>0.21, 0.23, 0.22</td>
<td>0.22</td>
<td>0.21-0.23</td>
<td>0.008</td>
</tr>
<tr>
<td>Goat Kidney</td>
<td>94, 95, 96</td>
<td>5.4, 5.5, 5.3</td>
<td>5.4</td>
<td>5.3-5.5</td>
<td>0.053</td>
</tr>
<tr>
<td>Goat Milk</td>
<td>101, 102, 103</td>
<td>0.055, 0.052, 0.037</td>
<td>0.048</td>
<td>0.037-0.055</td>
<td>0.010</td>
</tr>
</tbody>
</table>

(1) Corrected by % recovery from fresh fortification as shown by Table III for the fresh fortification concentration nearest that of the total $^{14}$C residues.
### TABLE III. SUMMARY TABLE FOR EXTRACTABILITY, ANALYSIS, AND ACCOUNTABILITY OF $^{14}$C-CGA-152005 TREATED SUBSTRATES USING METHOD AG-592

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SUBSTRATE</td>
<td>SAMPLE CODE</td>
<td>TOTAL RESIDUE (ppm)</td>
<td>EXTRACTABILITY</td>
<td>(1)</td>
<td>(2)</td>
<td>(3)</td>
<td>FINAL FRACTION</td>
<td>(4)</td>
<td>(5)</td>
</tr>
<tr>
<td>Poultry Lean Meat</td>
<td>1912600507</td>
<td>0 705</td>
<td>0.82, 0.82, 0.69</td>
<td>110</td>
<td>0.55, 0.53, 0.55</td>
<td>0.60, 0.58, 0.60</td>
<td>0.58, 0.56, 0.59</td>
<td>0.64, 0.62, 0.65</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>Egg</td>
<td>1912600533</td>
<td>0 326</td>
<td>0.24, 0.21, 0.24</td>
<td>72</td>
<td>0.083, 0.081, 0.082</td>
<td>0.096, 0.093, 0.095</td>
<td>0.085, 0.087, 0.088</td>
<td>0.099, 0.087, 0.088</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Goat Liver</td>
<td>G90480033</td>
<td>1 371</td>
<td>144, 1 38, 1.53</td>
<td>106</td>
<td>1.17, 1.21, 1.22</td>
<td>1.17, 1.21, 1.22</td>
<td>1.37, 1.44, 1.42</td>
<td>1.37, 1.44, 1.42</td>
<td>103</td>
<td></td>
</tr>
<tr>
<td>Goat Omental Fat</td>
<td>G90480024</td>
<td>0 058</td>
<td>0.060, 0.068, 0.090</td>
<td>148</td>
<td>0.064, 0.062, 0.068</td>
<td>0.080, 0.078, 0.086</td>
<td>0.065, 0.064, 0.067</td>
<td>0.082, 0.081, 0.084</td>
<td>142</td>
<td></td>
</tr>
<tr>
<td>Goat Round</td>
<td>G90480031</td>
<td>0 209</td>
<td>0.25, 0.24, 0.24</td>
<td>116</td>
<td>0.20, 0.20, 0.20</td>
<td>0.20, 0.21, 0.21</td>
<td>0.21, 0.22, 0.22</td>
<td>0.21, 0.23, 0.22</td>
<td>106</td>
<td></td>
</tr>
<tr>
<td>Goat Kidney</td>
<td>G90480026</td>
<td>5 048</td>
<td>4.7, 5.2, 5.6</td>
<td>103</td>
<td>4.7, 4.7, 4.6</td>
<td>4.7, 4.7, 4.6</td>
<td>5.4, 5.5, 5.3</td>
<td>5.4, 5.5, 5.3</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>Goat Milk</td>
<td>G90480012</td>
<td>0 069</td>
<td>0.054, 0.055, 0.055</td>
<td>79</td>
<td>0.025, 0.025, 0.015</td>
<td>0.035, 0.036, 0.22</td>
<td>0.039, 0.037, 0.026</td>
<td>0.055, 0.052, 0.017</td>
<td>70</td>
<td></td>
</tr>
</tbody>
</table>

(1) As determined by combustion analysis in metabolism studies.
(2) Determined by liquid scintillation counting of aliquots of the extract from Section III C.1 of AG-592.
(3) Average of three extractions shown in column D divided by the Total Residue shown in Column C, times 100.
(4) Determined by liquid scintillation counting of aliquots of the final fraction from Section II C.3.5.
(5) Corrected by % Recovery from fresh fortification as shown in Table III for the fresh fortification concentration nearest that of the total $^{14}$C residue.
(6) Determined by high performance liquid chromatography.
(7) Average of Column I, divided by Column C, and multiplied by 100.