Thidiazuron/R60
A61009

Title

Supplement 1 to: "At-Harvest Thidiazuron-Derived Residues in or on Processed Cottonseeds Following Two Treatments with DROPP® 50WP at Exaggerated Rates, 7 Days PHI, USA, 1994"

Author

Original study: Lee E. Williams
This Supplement: Lee E. Williams

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Data Requirement

Guideline Subdivision O
Guideline 171-4(l)

Report Date

Original study: February 23, 1996
This supplement: October 17, 1996

Performing Laboratory

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This supplement: To be assigned

Total Number of Pages 59

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

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

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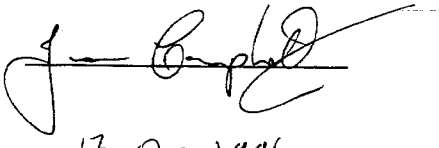
Company Agent: James K. Campbell, Ph.D.
Manager, Residue Chemistry

Signature: 

Date: 17-Oct-1996

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The undersigned, hereby declare that the work to which this report amendment refers was performed according to the procedures herein described and this report amendment provides an accurate record of the results obtained. The amended study was conducted in accordance with the Good Laboratory Practice Standards as specified in 40 CFR 160.

Study Director: _____ 10/17/96
Lee E. Williams, Ph.D. — Date
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Sponsor: _____ 17-Oct-1996
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Manager, Residue Chemistry

Submitter: _____ 23-Oct-1996
Date

QUALITY ASSURANCE STATEMENT

This study was inspected and the findings reported to the facility management and to the study director on the listed dates:

Inspection Date	Date Reported to Study Director	Date Reported to Facility Management
May 21, 1996	May 23, 1996	May 24, 1996
May 29, 1996	May 29, 1996	May 29, 1996
May 30, 1996	May 30, 1996	June 6, 1996
July 31, 1996	August 1, 1996	August 12, 1996

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Senior Quality Assurance Officer

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At-Harvest Thidiazuron-Derived Residues in or on Processed Cottonseeds Following Two Treatments with DROPP® 50WP at Exaggerated Rates, 7 Days PHI, USA, 1994

Amendment No. 1.

Reason for amendment:

I. In Appendix V of the original report, transcription errors were noted after the report was issued. In addition, chromatograms for treated samples were omitted and two chromatograms did not photocopy adequately.

II. An improved method was developed which was considered to be more suitable than the original method for tolerance enforcement purposes.

III. Cottonseed samples were re-analyzed using the revised method to demonstrate that both methods gave essentially the same result. Gin trash samples not included in the original report were analyzed and reported.

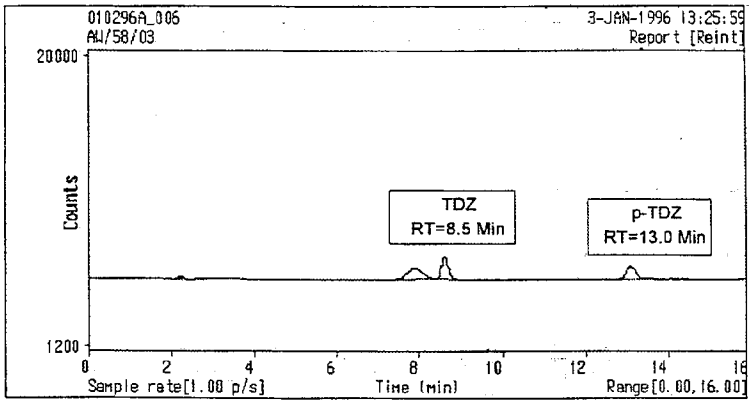
IV. Radiovalidation of the revised analytical method is included in this amendment.

I. Revised Appendix V

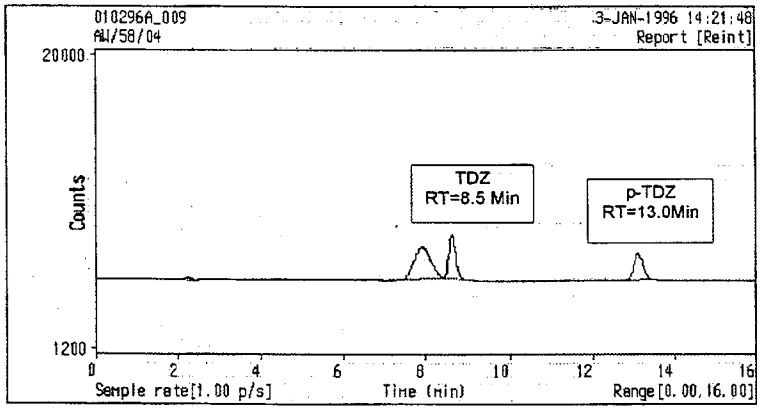
Figures 1 through 13 replace Appendix V in the original report.
Registration Reference: Thidiazuron/R60/A55693.

The retention time of both analytes can vary over a significant range. Refined oil samples for example have a clean baseline and the retention time can be made very short in order to save time. Cottonseed samples produce more complex chromatograms and the retention times have to be adjusted to give adequate resolution of the analytes. The standards for each set of samples are run under the same conditions as the samples.

Figure 1 Chromatograms of Standards

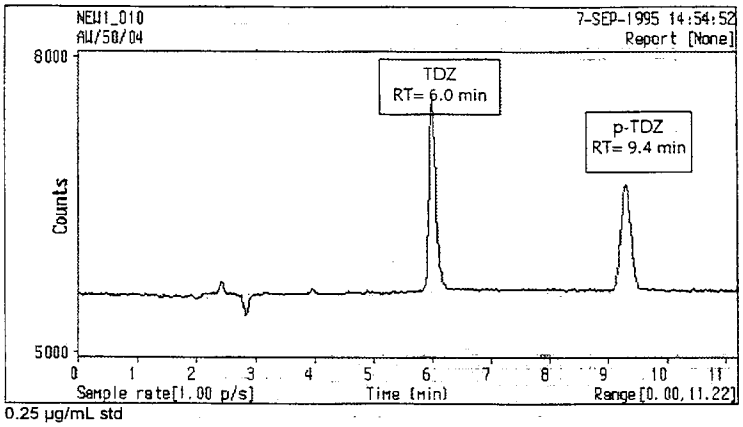


0.25 µg/mL standard

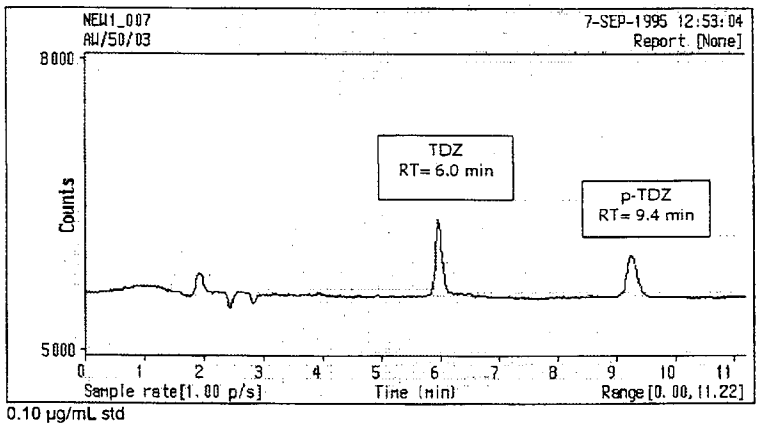


0.50 µg/mL standard

Figure 2 Chromatograms of Standards

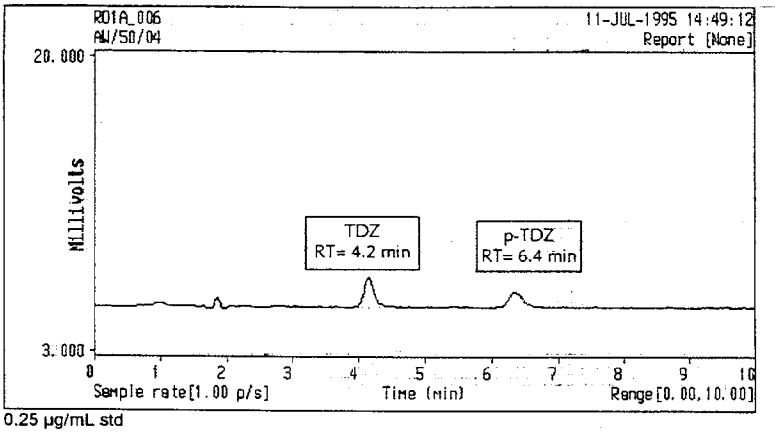


0.25 µg/mL std

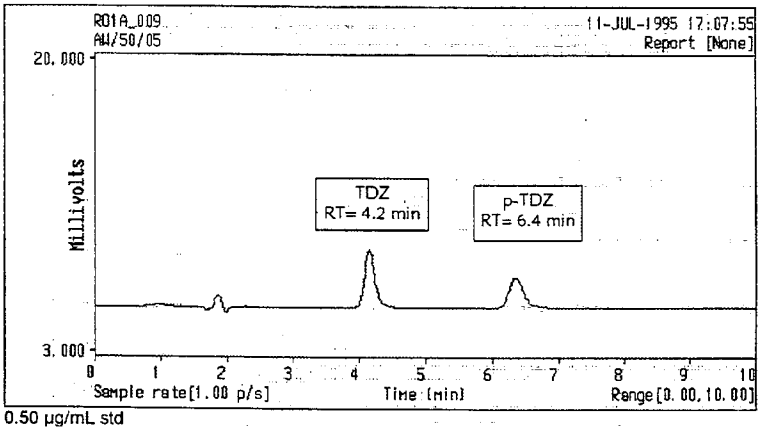


0.10 µg/mL std

Figure 3 Chromatograms of Standards

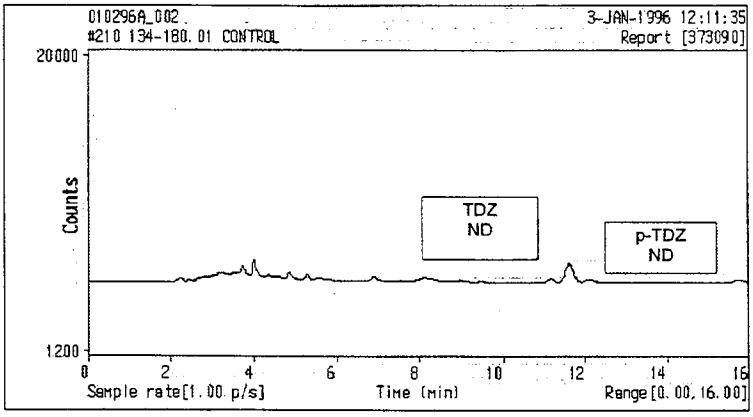


0.25 µg/mL std

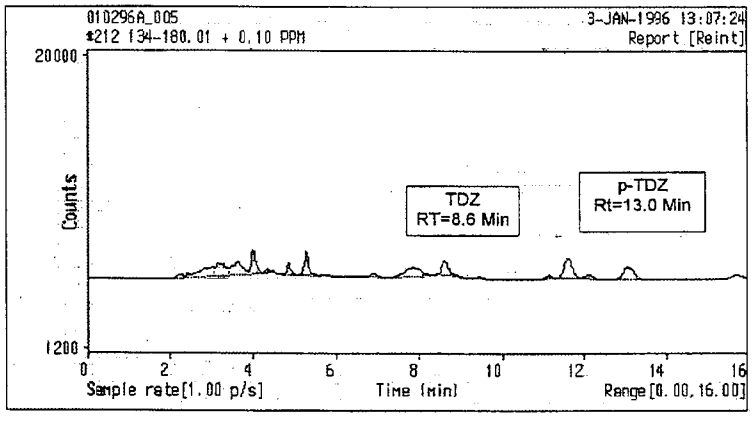


0.50 µg/mL std

Figure 4 Chromatograms of Control and Fortified Cottonseed

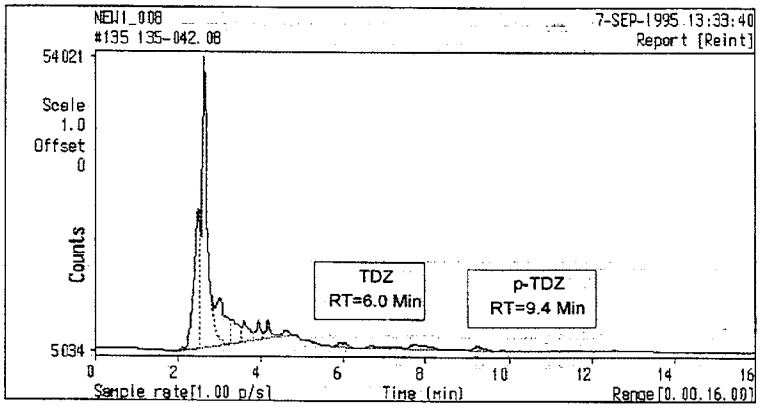


Control cottonseed (134-180.01)
<0.05 TDZ, <0.05 p-TDZ

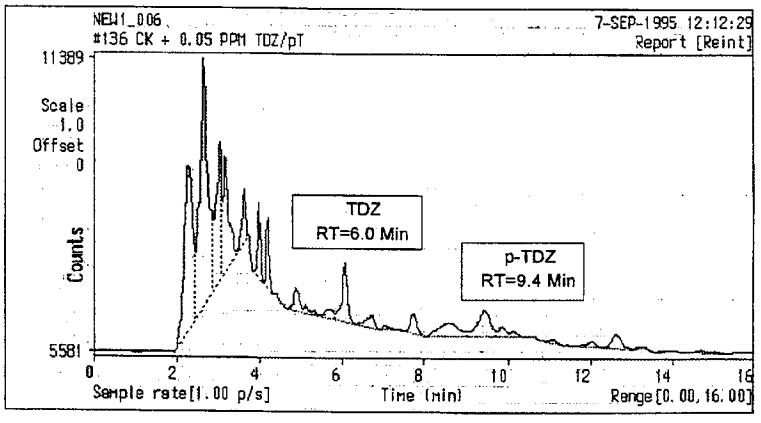


Control cottonseed (134-180.01) + 0.10 ppm
Amount Found: 0.067 ppm TDZ, 0.089 p-TDZ
Amount Added: 0.100 ppm
Recovery : 66% TDZ, and 89% p-TDZ

Figure 5 Chromatograms of Control and Fortified Cotton

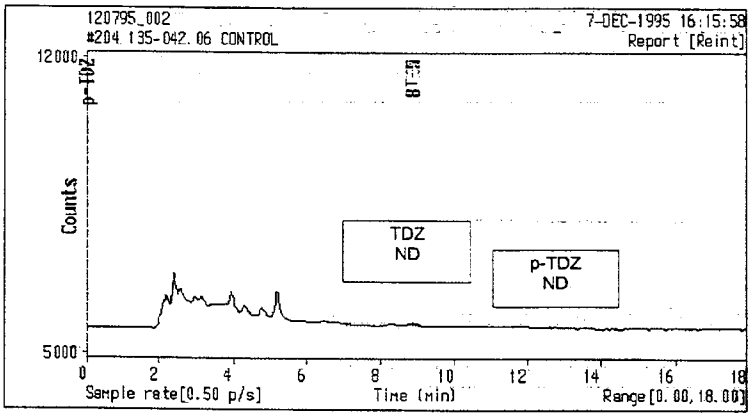


Control cotton (135-042.08)
<0.05 ppm TDZ, <0.05 ppm p-TDZ

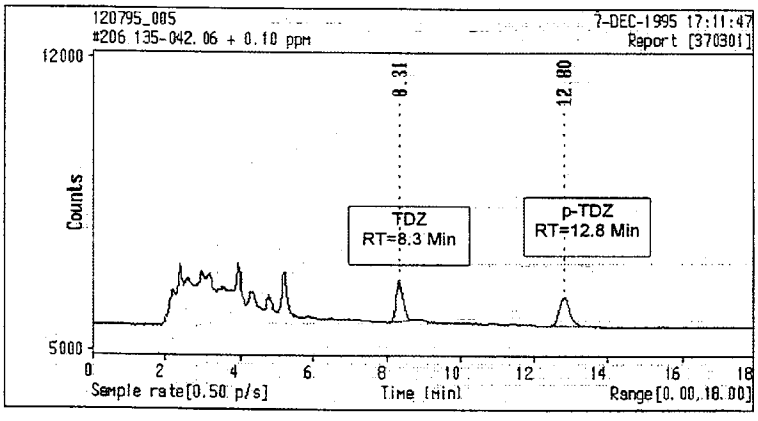


Control cotton (135-042.08) + 0.05 ppm
Amount Found: 0.045 ppm TDZ, 0.0502 p-TDZ
Amount Added: 0.05 ppm
Recovery : 91% TDZ, and 101% p-TDZ

Figure 6 Chromatograms of Control and Fortified Cottonseed Meal

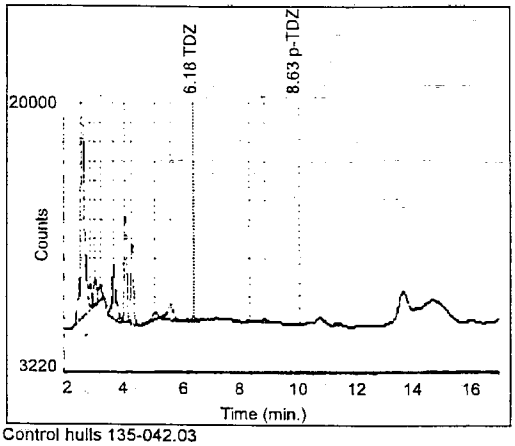


Control cottonseed meal (135-042.06)
<0.05 ppm TDZ, <0.05 ppm p-TDZ

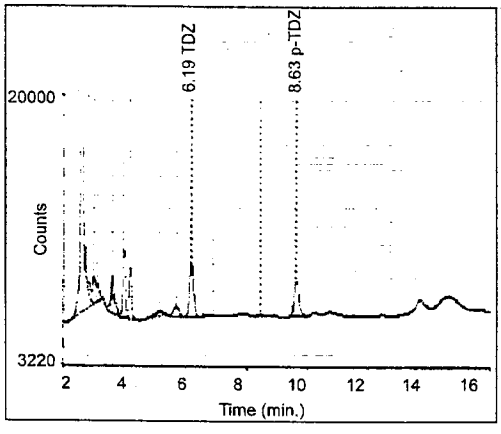


Control cottonseed meal (135-042.06) +0.10 ppm
Amount Found: 0.074 ppm TDZ, 0.089 p-TDZ
Amount Added: 0.10 ppm
Recovery : 74% TDZ, and 89% p-TDZ

Figure 7 Chromatograms of Control and Fortified Cottonseed Hulls

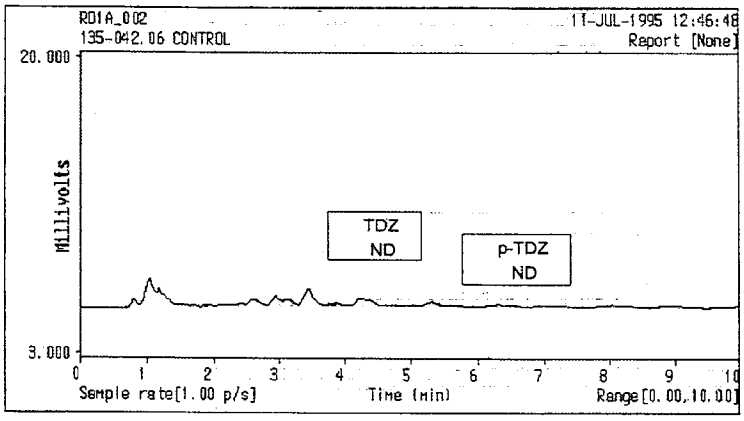


Control hulls 135-042.03
<0.05 ppm TDZ
<0.05 ppm p-TDZ

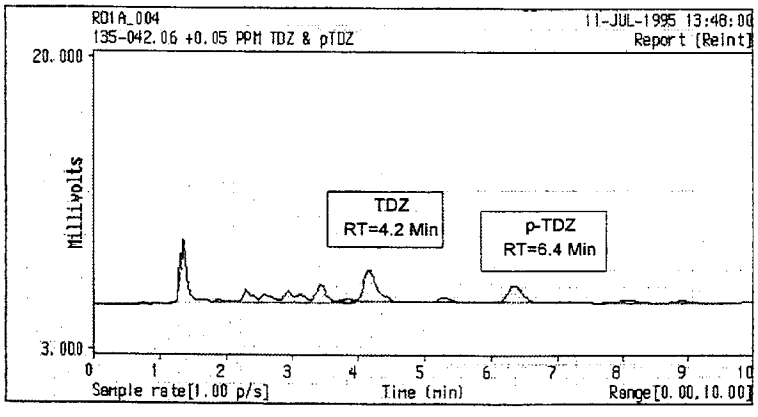


Control hulls 135-042.03 + 0.50 ppm TDZ and p-TDZ
Amount found: 0.3939 ppm TDZ, 0.4527 ppm p-TDZ
Amount added: 0.50 ppm TDZ, 0.50 ppm p-TDZ
Recovery: 71% TDZ, 91% p-TDZ

Figure 8 Chromatograms of Control and Fortified Refined Oil

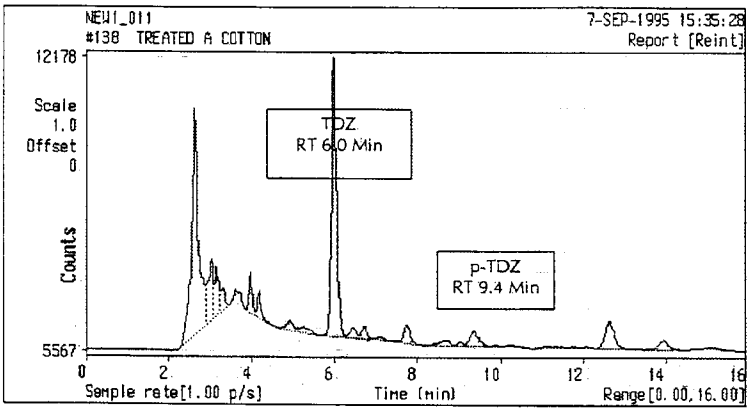


Control Refined oil (135-042.06)
<0.05 ppm TDZ, <0.05 ppm p-TDZ

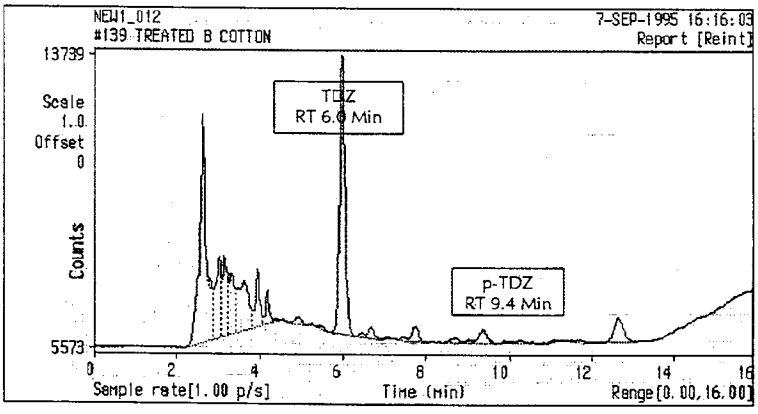


Control Refined oil (135-042.06) + 0.05 ppm
Amount Found: 0.054 ppm TDZ, 0.056 p-TDZ
Amount Added: 0.05 ppm
Recovery : 82% TDZ, and 98% p-TDZ

Figure 9 Chromatograms of treated cotton samples

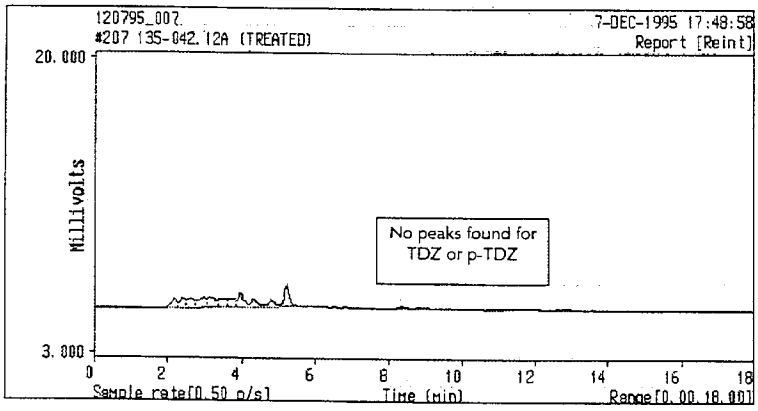


Cotton treated (135-042.16)A
Amount Found: 0.62 ppm TDZ, 0.062 ppm p-TDZ (Results uncorrectd for recovery value.)

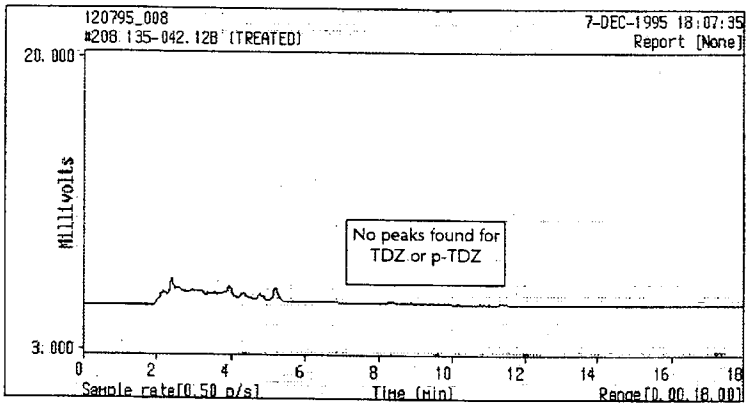


Cotton treated (135-042.16)B
Amount Found: 0.77 ppm, 0.072 ppm p-TDZ (Results uncorrectd for recovery value.)

Figure 10 Chromatograms of Treated Cottonseed Meal Sample

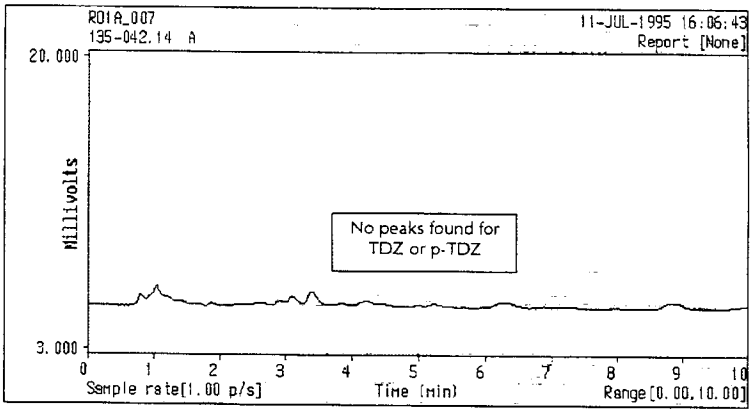


Cottonseed meal 135-042.12A (Treated)
Amount Found: <0.05 ppm TDZ, <0.05 ppm p-TDZ

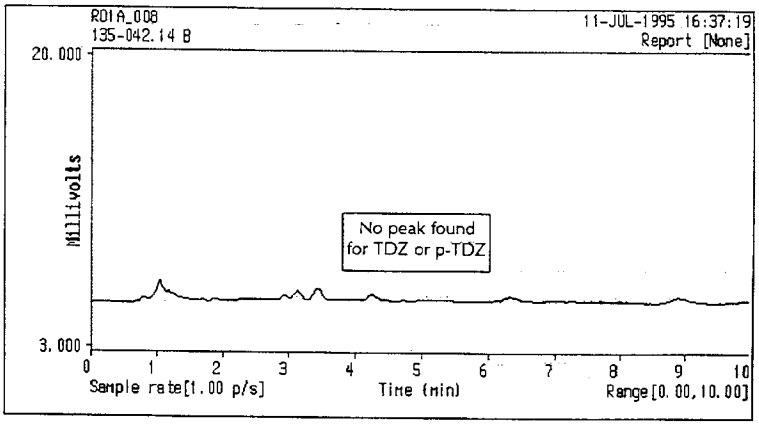


Cottonseed meal 135-042.12B (Treated)
Amount Found: <0.05 ppm TDZ, <0.05 ppm p-TDZ

Figure 11 Chromatograms of Treated Cottonseed Refined Oil Samples

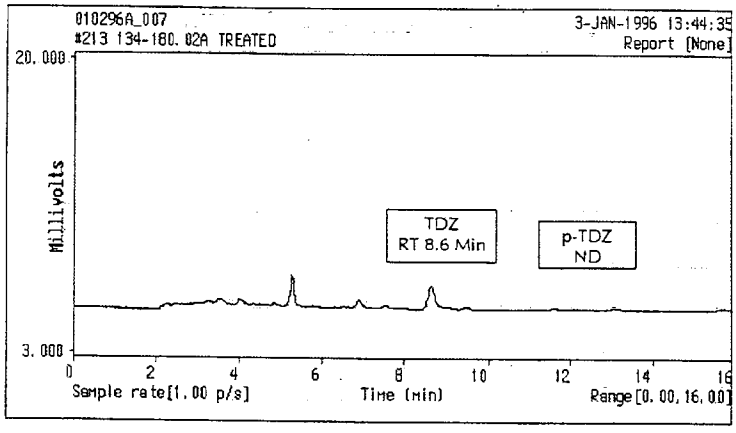


Cottonseed refined oil treated (135-042.14)A
Amount Found: <0.05 ppm TDZ, <0.05 ppm p-TDZ

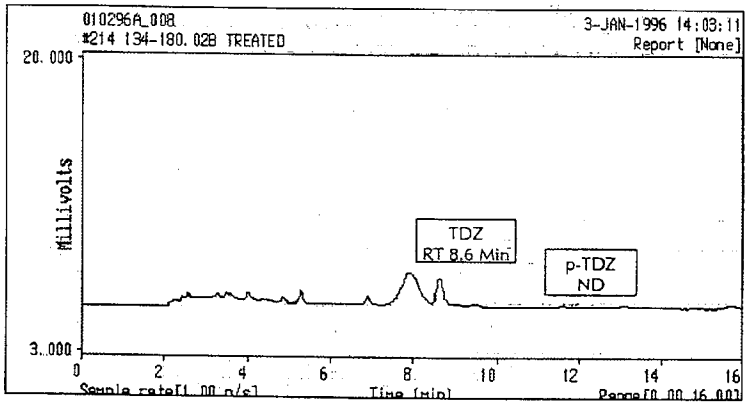


Cottonseed refined oil treated (135-042.14)B
Amount Found: <0.05 ppm TDZ, <0.05 ppm p-TDZ

Figure 12 Chromatograms of Treated Cottonseed Samples



Cottonseed treated (134-180.02)A
Amount Found: 0.090 ppm TDZ, <0.05 ppm p-TDZ (Results uncorrectd for recovery value.)



Cottonseed treated (134-180.02)B
Amount Found: 0.104 ppm TDZ, <0.05 ppm p-TDZ (Results uncorrectd for recovery value.)

II. Revised Analytical Method (AW/02/96)

This method AW/02/96 will be used as an additional method to AW/02/95. Data submitted from AW/02/96 is additional data generated for cottonseed and new data for the gin trash analysis.

1. SCOPE

This method is suitable for the determination of extractable residues of the cotton defoliant, thidiazuron (TDZ), and its primary degradation product, photo-thidiazuron (photo-TDZ), in cottonseed and gin trash. The limits of quantitation for this procedure have been set at 0.05 ppm for cottonseed and 0.50 ppm for gin trash.

2. SUMMARY

The extraction has been separated into two schemes: one for cottonseed and one for gin trash.

Extractable residues of thidiazuron and photo-thidiazuron are removed from cottonseed by blending with acetonitrile. The resulting extract is filtered, concentrated by rotary evaporation, and partitioned with hexane. The acetonitrile extract is then rotary evaporated to dryness. Residues of TDZ and photo-TDZ are reconstituted in a mixture of 70/30 (v/v) ethyl acetate/cyclopentane. This extract is filtered, loaded onto a GPC Optima prep system, and eluted with a mixture of 70/30 (v/v) ethyl acetate/cyclopentane. The fraction containing TDZ and photo-TDZ residues is collected and rotary evaporated to dryness. These residues are reconstituted in a mixture of 60/40 (v/v) acetonitrile/deionized water and quantified by High Performance Liquid Chromatography with ultraviolet detection at 290 nm.

Extractable residues of thidiazuron and photo-thidiazuron are removed from gin trash by blending with 80/20 (v/v) acetonitrile/deionized water. The resulting extract is filtered, concentrated by rotary evaporation, diluted with saturated sodium chloride, and extracted with dichloromethane. The dichloromethane extract is dried through a sodium sulfate pad and then rotary evaporated to dryness. The residue is reconstituted in acetonitrile and partitioned with hexane. The acetonitrile extract is rotary evaporated to dryness. Residues of TDZ and photo-TDZ are reconstituted in methanol and cleaned up by column chromatography on basic aluminum oxide using methanol as the eluting solvent. The methanol eluate is rotary evaporated to dryness and the residue is reconstituted in dichloromethane. Following further clean-up by solid phase extraction, TDZ and photo-TDZ residues are quantified by High Performance Liquid Chromatography with ultraviolet detection at 290 nm.

3. APPARATUS

(equivalents may be substituted)

- Pint Mason jars
- Glass fiber filter paper, Whatman AH-934
- Separatory funnels, 250-mL, with stoppers
- Boiling flasks, 125-, 250-, and 500 mL
- Graduated cylinders, 10-, 50-, and 100-mL TD
- Beakers, 250-mL
- Class A volumetric pipets, 0.5-, 1.0-, 4.0- and 10.0-mL
- Vacuum adapters, glass, 24/40
- Volumetric flasks, 5- and 10-mL
- Büchner funnels, 9 cm
- Sorval Omni Mixer (Model #17105) and blade assemblies
- Ultrasonic Bath Cleaner
- Transfer Pasteur pipets, flint glass, 5.75" and 9"
- Powder funnels, long-stem, 75 mm
- Fiber glass sliver, 8 micron, Pyrex
- Plastic syringes, luer lock, 3- and 10-mL
- Aluminum oxide 60, Active Basic (Activity I), 70-230 mesh ASTM, EM Science
- Chromatography columns, glass, with reservoir, Krackler Scientific (Cat. No. 17810-11300)
- Solid phase extraction (SPE) columns, MegaBond Elut® Silica, 10 gram/60 mL reservoir, Varian
- J. T. Baker SPE-12G column processor, consisting of 12-port vacuum manifold
- Analytical column, Prism RP-18, 250 mm x 4.6 mm i. d., Keystone Scientific, Inc.
- LC-ABZ Supelguard column, 2 cm x 4.6 mm i.d., Supelco
- HPLC System, UV @ 290 nm
- 13 mm syringe filters, Acrodisc® LC13 PVDF, 0.45 µm pore size
- Micropipettes, 250-µL (optional)
- Autovap AS-2000 Sample Processing System, O. I. Analytical
- GPC Optima Teflon prep column 400, mm x 16 mm, i.d., O. I. Analytical

4. REAGENTS

(equivalents may be substituted)

- Acetonitrile, HPLC grade
- Cyclopentane, HPLC grade
- Deionized water

- Dichloromethane, HPLC grade
- Ethyl Acetate, HPLC grade
- Hexane, pesticide grade
- Methanol, HPLC grade
- Saturated sodium chloride solution
- Sodium sulfate, granular anhydrous, ACS reagent
- 80/20 (v/v) Acetonitrile/Deionized water
- 60/40 (v/v) Acetonitrile/Deionized water
- 70/30 (v/v) Ethyl acetate/cyclopentane
- 1% Methanol in Dichloromethane
- 4% Methanol in Dichloromethane
- Analytical standards of known purity for thidiazuron and photo-thidiazuron

5. PROCEDURE

5.1 Cottonseed Extraction

5.1.1 Weigh 20 grams of a representative cottonseed sample into a pint Mason jar. Fortify the recovery samples at this point (see Section 6.3).

5.1.2 Add 150 mL of acetonitrile, and blend for 10 minutes at medium speed with the Omni mixer. Filter the sample under suction through a Büchner funnel equipped with glass fiber filter paper into a 500-mL boiling flask. Rinse the Mason jar with 50 mL of acetonitrile. Filter the rinse. Use the bottom of the Mason jar to firmly press the retained cottonseed in the Büchner funnel, collecting the remaining filtrate.

5.1.3 Rotary evaporate the acetonitrile extract to a volume of 40 to 50 mL at 40 °C (± 5 °C) under reduced pressure.

5.1.4 Transfer the acetonitrile extract from the 500-mL boiling flask to a 250-mL separatory funnel. Rinse the boiling flask with 50 mL of hexane, and transfer the rinse to the separatory funnel containing the acetonitrile extract. Shake the separatory funnel for one minute. Allow the layers to separate. Drain the lower (acetonitrile) layer into a clean 250-mL boiling flask.

5.1.5 Rinse the 500-mL boiling flask with 25 mL of acetonitrile. Transfer the rinse to the 250-mL separatory funnel containing the hexane. Shake the separatory funnel for one minute. Allow the layers to separate. Combine the lower (acetonitrile) extract with the initial extract. Rotary evaporate the combined extracts to dryness at 40 °C (± 5 °C) under reduced pressure.

5.1.6 Reconstitute the residue in 5 mL of 70/30 (v/v) ethyl acetate/cyclopentane. Filter this extract through an Acrodisc® LC13 PVDF syringe filter (fitted on a 10-mL syringe) into a clean 125-mL boiling flask. Proceed to GPC clean-up (Section 5.3).

5.2 Gin Trash Extraction

5.2.1 Weigh 10 grams of a representative gin trash sample into a pint Mason jar. Fortify the recovery samples at this point (see Section 6.3).

5.2.2 Add 150 mL of 80/20 (v/v) acetonitrile/deionized water, and blend for 10 minutes at medium speed with the Omni mixer. Filter the sample under suction through a Büchner funnel equipped with glass fiber filter paper into a 500-mL boiling flask. Rinse the Mason jar with 2 x 50 mL of acetonitrile. Filter the rinses. Use the bottom of the Mason jar to press the retained gin trash in the Büchner funnel, collecting the remaining filtrate.

5.2.3 Rotary evaporate the extract to a volume of less than 50 mL at 40 °C (± 5 °C) under reduced pressure. NOTE: Due to the presence of fine particulates (the quantity of which is matrix dependent) in the filtrate, solvent bumping will occur. Close monitoring during rotary evaporation is required.

5.2.4 Add 50 mL of saturated sodium chloride to the extract in the boiling flask. Transfer the resulting aqueous extract to a 250-mL separatory funnel. Extract with dichloromethane (3 x 50 mL). (Note: The requisite dichloromethane volumes should be used to rinse the boiling flask prior to addition to the separatory funnel.) Drain the dichloromethane phases through a sodium sulfate pad (approximately 90 grams of sodium sulfate in a glass wool-plugged powder funnel) into a clean 500-mL boiling flask. (Note: In the initial extraction, the dichloromethane phase may be the upper layer. In this case, return the lower (aqueous) phase to the original 500-mL boiling flask, collect the upper (dichloromethane) phase as described, and transfer the aqueous phase back to the separatory funnel.) Rinse the sulfate pad with 25 mL of dichloromethane. Discard the aqueous phase.

5.2.5 Rotary evaporate the combined dichloromethane extracts to dryness at 40 °C (± 5 °C) under reduced pressure.

5.2.6 Reconstitute the residue in 100 mL of acetonitrile. (Sonication may be necessary.) Transfer the extract to a clean 250-mL separatory funnel. Rinse the boiling flask with 100 mL of hexane, and transfer the rinse to the separatory funnel containing the acetonitrile extract. Shake the separatory funnel for one minute. Allow the layers to separate. (Note: A slight emulsion may form. This can be dissipated by slight stirring of the biphasic mixture with a long-stemmed Pasteur pipet.) Drain the lower (acetonitrile) phase into a clean 500-mL boiling flask. Rinse the original 500-mL boiling flask with 50 mL of acetonitrile. Transfer the rinse to the separatory funnel containing the hexane. Shake the separatory funnel for one minute. Allow the layers to separate. Combine the lower (acetonitrile) phase with the initial extract. Discard the hexane.

5.2.7 Rotary evaporate the combined acetonitrile extracts to dryness at 40 °C (± 5 °C) under reduced pressure. Reconstitute the residue in 10 mL of methanol. Proceed to Section 5.4 for clean-up.

5.3 GPC Optima Teflon Prep Column Clean-up for Cottonseed

In order to perform sample clean-up by GPC, a column dump (sample clean-up) time and a column collect time for the analyte(s) of interest must be determined. A column wash time may also be required. To determine the appropriate dump and collect times for quantitative recovery of the analyte(s), proceed as follows:

Instrument Settings:

GPC ultrasonic pre-load time: 0.02 seconds
GPC flow rate: 3.5 mL/min
Detector wavelength: 280 nm
Chart speed: 1.0 cm/min
Output voltage: 1 mV

5.3.1 Transfer 100 µL of the 1000 µg/mL standard stock solution to a 125-mL boiling flask. Dilute with 5 mL of 70/30 (v/v) ethyl acetate/cyclopentane.

5.3.2 Load the diluted standard into one of the test tubes on the GPC sample prep station.

5.3.3 Connect the column outlet line to the inlet line of the UV detector. Monitor the UV absorbance via a strip chart recorder.

5.3.4 Dump the column eluate to the UV detector for 30 minutes. This is the default time. The analyst may adjust this time as needed.

5.3.5 Monitor the elapsed time to the start of analyte elution. This time is the dump or sample clean-up time. Eluate collected during this time is discarded.

5.3.6 Monitor the start and end times for detection of the analyte(s). This span of time is the collection time, and eluate collected during this time is retained for analysis.

5.3.7 Once the dump and collection times are established, program these times into the GPC Autoprep 1000 module. Typical times are 10.30 minutes and 6.30 minutes, respectively. Set the wash time at 5 minutes. Allow a window on either side of the collection period to accommodate any slight drift in retention time.

5.3.8 Load the samples from step 5.1.6 into the glass test tubes located on the GPC unit.

5.3.9 Index through the program to the **start with sample** prompt, enter the appropriate beginning sample number. Use 1 through 23. At the **terminal sample** prompt, enter the appropriate ending sample number. Use 1 through 23.

5.3.10 Disconnect the outlet line of the column from the UV detector. Reconnect this line to the GPC AS-2000 sample processing system.

5.3.11 Index through the Autoprep 1000 module's program until the **push run** prompt appears. Press the run button and sample clean-up will begin. Note that the GPC uses only 2.5 mL of the original 5-mL sample for clean-up.

5.3.12 Rotary evaporate the collected extract to dryness at 40 °C (± 5 °C) under reduced pressure.

5.3.13 Reconstitute residues of TDZ and photo-TDZ in 4 to 10 mL of 60/40 (v/v) acetonitrile/deionized water. Filter the samples through an Acrodisc® LC13 PVDF syringe filter prior to HPLC analysis.

5.4 Column Clean-up for Gin Trash

5.4.1 *Prepare a basic aluminum oxide column.* Using a glass rod, push a very small plug of glass wool to the bottom of a clean glass chromatography column (with an inner diameter of approximately 2 cm). Add 15 g of basic aluminum oxide to the column. Level the aluminum oxide by gently tapping the sides of the glass column. Carefully pour 50 mL of methanol into the column. Open the stopcock of the column and drain the methanol, by gravity, into a beaker. While the methanol is draining through the column, wash any aluminum oxide on the sides of the column onto the packing with methanol, using a wash bottle or a Pasteur pipet. Using a glass rod, push another small plug of glass wool to the top of the column. Allow the methanol to drain through the column until a small layer (approximately 1 mm) remains above the top of the packing. Close the stopcock. Do not allow the column to go dry.

NOTE on **column characterization**: An elution profile should be performed on each batch of aluminum oxide prior to use. The volume of methanol needed to elute the TDZ and photo-TDZ residues can vary depending on the manufacturer and/or batch of the aluminum oxide. The elution profile must be established using a fortified extract as the plant material in the extract influences the retention time of the analytes. Fortify a control acetonitrile extract of gin trash (from Step 5.2.6) with 10 µg of both TDZ and photo-TDZ. Rotary evaporate the extract to dryness at 40 °C (± 5 °C) under reduced pressure. Reconstitute the residue in 10 mL of methanol and load onto the aluminum oxide column. Wash the column with 20 mL of methanol. Collect both the load and the wash in a single 125-mL boiling flask. Elute the column with an additional 5 x 30 mL of methanol, collecting each fraction in a separate 125-mL boiling flask. Rotary evaporate each fraction, including the combined load/wash, to dryness at 40 °C (± 5 °C) under reduced pressure. Reconstitute each fraction in 5-10 mL of

60/40 (v/v) acetonitrile/deionized water. Filter each reconstituted fraction through an Acrodisc® LC13 PVDF syringe filter and analyze by HPLC/UV. Compare the responses with those of known standards. Recoveries should be greater than 90%. If necessary, the volume of methanol used for elution may be adjusted to obtain these recoveries.

5.4.2 Pipet the methanolic extract from Step 5.2.7 onto the top of the packing. Open the stopcock and allow the eluate to flow by gravity into a clean 250-mL boiling flask. Close the stopcock when the solvent level reaches the top of the packing.

5.4.3 Rinse the original boiling flask with 20 mL of methanol. Carefully pour this rinse onto the top of the packing. Open the stopcock and collect the eluate, combining it with the first collection. Close the stopcock when the solvent level reaches the top of the packing.

5.4.4 Carefully pour 120 mL of methanol onto the top of the packing (this volume may be adjusted accordingly based on the elution profile from Step 5.4.1). Open the stopcock and collect the eluate, combining it with the previous collections. Close the stopcock when the solvent level reaches the top of the packing.

5.4.5 Rotary evaporate the combined extracts to dryness at 40 °C (± 5 °C) under reduced pressure. Reconstitute the residue in 10 mL of dichloromethane and proceed to clean-up by solid phase extraction (Step 5.4.6).

5.4.6 Place a 60-mL MegaBond Elut® Silica SPE column onto a J. T. Baker vacuum manifold for each sample to be analyzed.

NOTE on **column characterization**: An elution profile should be performed on each batch of SPE silica columns prior to use. The wash and elution patterns can vary depending on the manufacturer and/or batch. The column profile should be performed on a fortified control gin trash sample. Fortify a control methanolic extract of gin trash (from Step 5.4.4) with 10 µg of both TDZ and photo-TDZ. Rotary evaporate the extract to dryness at 40 °C (± 5 °C) under reduced pressure.

Reconstitute the residue in 10 mL of dichloromethane and load onto a conditioned (see Step 5.4.7) SPE column. Drain the dichloromethane from the column into a beaker. Rinse the flask with 20 mL of dichloromethane. Drain the rinse from the column into the beaker. Discard both the sample load and rinse. Elute the column with 4 x 25 mL of a solution of 1% methanol in dichloromethane followed by 4 x 25 mL of a solution of 4% methanol in dichloromethane, collecting each fraction in a separate 125-mL flask. Rotary evaporate each fraction to dryness at 40 °C (± 5 °C) under reduced pressure. Reconstitute each fraction in 5 - 10 mL of 60/40 (v/v) acetonitrile/deionized water. Filter each reconstituted fraction through an Acrodisc® LC13 PVDF syringe filter and analyze by HPLC/UV. Compare the responses with those of known standards. Recoveries should be greater than 90%. If necessary, adjust the volume of the solution of 4% methanol in dichloromethane used for elution to obtain these recoveries. According to the column profile, set the appropriate wash and elution volumes for TDZ and photo-TDZ recovery. Typically, these values are a wash volume of 80 mL of 1% methanol in dichloromethane and an elution volume of 20 mL of 1% methanol in dichloromethane followed by 80 mL of 4% methanol in dichloromethane.

5.4.7 Condition each column with one column volume of 4% methanol in dichloromethane, followed by two column volumes of dichloromethane. Do not allow the column to go dry prior to sample application.

5.4.8 Transfer the reconstituted dichloromethane extract (from Step 5.4.5) onto the column. Drain the dichloromethane from the column into a beaker. Do not allow the column to go dry prior to rinse application. Rinse the boiling flask with 20 mL of dichloromethane and load onto the column. Drain the rinse from the column into the beaker. Discard both the sample load and rinse.

5.4.9 Wash the column with 80 mL of 1% methanol in dichloromethane. Drain the wash from the column into a beaker. Discard the wash.

5.4.10 Elute the column with 20 mL of 1% methanol in dichloromethane followed by 80 mL of 4% methanol in dichloromethane. Collect the eluates in a single 250-mL boiling flask. Rotary evaporate the combined eluates to dryness at 40 °C (± 5 °C) under reduced pressure.

5.4.11 Reconstitute the residue in an appropriate volume of 60/40 (v/v) acetonitrile/deionized water. Filter though an Acrodisc® LC13 PVDF syringe filter prior to HPLC analysis.

6. HPLC DETERMINATION OF THIDIAZURON AND PHOTO-THIDIAZURON

6.1 Preparation of Analytical Standards

6.1.1 Prepare a stock solution containing a nominal concentration of 1000 µg/mL of thidiazuron and photo-thidiazuron in methanol. Make dilutions with methanol to yield a nominal concentration of 100 µg/mL for each compound. Prepare a mixed standard solution containing a nominal concentration of 10 µg/mL of thidiazuron and photo-thidiazuron in methanol. From this mixed standard, make serial dilutions with 60/40 (v/v) acetonitrile/deionized water to yield HPLC calibration standards of 0.05, 0.10, 0.25, 0.50, 1.0, and 2.0 µg/mL of TDZ and photo-TDZ in 60/40 (v/v) acetonitrile/deionized water.

6.1.2 Standardize the HPLC/UV under the conditions in Appendix II by making 30-µL injections of the calibration standard solutions interspersed with the samples.

6.1.3 Construct a least squares standard curve of detector response versus standard concentration. (See Figures 15 and 16.) This method was validated using peak height; however, peak areas may be used if preferred.

6.2 Detection of Sample Residues

6.2.1 Inject a 30-µL aliquot of the extracts from 5.3.14 and 5.4.11 into the HPLC/UV system under the conditions stated in Appendix II. An injection volume of 30 µL was found to give satisfactory results. Variations in equipment or sample characteristics may require different injection volumes. Make dilutions as necessary to maintain the response within the range of the standard curve.

6.2.2 Compare the peak height of the unknown sample with the standard curve. Determine the concentrations of the analytes in the injected aliquot as described in 6.2.3.

6.2.3 Calculate the total residue concentration of each analyte by equation 1.

$$ppm\ analyte = \frac{\{[(y - b) / M]\}}{C} \times \frac{1}{V} \times \frac{1}{R} \quad \text{(Eq. 1)}$$

Where:
- y = Peak height for analyte (cts*.)
- b = Y-Intercept of the standard calibration curve (cts.)
- M = Slope of the standard calibration curve (cts/µg/mL)
- C = Crop solvent ratio expressed as grams crop per mL at injection (g/mL)
- V = Injection volume (µL)
- R = Recovery factor determined using fortified control samples where recovery correction is expressed as a decimal (i.e. 80% = 0.80 etc.)

*Cts = integrator counts

6.3 Fortification Experiments

6.3.1 Untreated control samples may be analyzed using the procedures described to verify that any endogenous substances in the samples do not interfere with the final determination of thidiazuron and photo-thidiazuron.

6.3.2 Recovery is determined by analyzing fortified control samples in conjunction with each sample set. Cottonseed samples are fortified prior to analysis with 1.0 µg or greater of thidiazuron and photo-thidiazuron. For example, add 100 µL of a 10 µg/mL standard solution of thidiazuron and photo-thidiazuron in methanol to 20 g of cottonseed for a 0.05 ppm matrix spike. Gin trash samples are fortified prior to analysis with 5.0 µg or greater of thidiazuron and photo-thidiazuron. Chromatograms of control and fortified control samples are presented in Figures 16 to 21.

6.3.3 Analyze the control and recovery samples by the same procedure described for unknown samples.

6.3.4 Calculate the final concentration values for the control and recovery samples according to equation 1.

6.3.5 Correct the results of the recovery samples by subtracting any apparent residues in the controls. Correct the concentrations of the treated samples for recovery results less than 100%.

7. DISCUSSION

Control and low recovery cottonseed samples provide best results when dissolved in 4 mL of the injection solvent (60/40 (v/v) acetonitrile/deionized water). High recovery samples give best results when dissolved in 10 mL of the injection solvent.

Control and low recovery gin trash samples provide best results when dissolved in 10 mL of the injection solvent (60/40 (v/v) acetonitrile/deionized water). High recovery samples give best results when dissolved in 100 mL of the injection solvent.

This method is suitable for tolerance enforcement purposes. For cottonseed, the background is clean, with no peaks close enough to the retention time of either analyte to cause identification ambiguity or integration problems. In the case of gin trash, the chromatogram is more complex and interferences are present at the retention time of both analytes. In practice, this should not matter since residue levels in treated samples will be high and the interference peaks will be negligible in relation to the analyte peaks.

The limit of quantitation for this method in cottonseed is 0.05 ppm for both analytes. This is based on the recoveries achieved at that concentration. Taking into account all of the 0.05 ppm fortifications analyzed in this piece of work, the recoveries ranged from 71% to 116% which is close to the acceptable limit. In the case of gin trash, the LOQ is 0.5 ppm. The interference from the background makes a lower LOQ impossible with this procedure.

A set of six samples can be analyzed by one person in two working days, assuming that all materials and equipment are available.

The most critical steps in the method are the column characterizations. Unless the elution patterns of the GPC, aluminum oxide, and silica columns are accurately established, significant losses of analyte may occur.

Thidiazuron is photo-sensitive; therefore, if processing has to be interrupted, the extracts should be stored cold and in the dark. However, if photodegradation does occur, the residue will still be detected as the photo-thidiazuron. Processing may be interrupted at any stage.

Table 1 HPLC Operating Conditions for the Determination of Thidiazuron and Photo Thidiazuron

Instrument	Hitachi L-6200A Intelligent Pump or equivalent Perkin-Elmer LC 90 Variable UV Detector or equivalent Hitachi AS-2000 Autosampler or equivalent PE Nelson Data Collection System (900 Series Interface with Access*Chrom software) or equivalent
Column	Prism RP-18 analytical column, 250 mm x 4.6 mm i.d., Keystone LC-ABZ Supelguard column, 2 cm x 4.6 mm i.d., Supelco
Mobile Phase	60/40 (v/v) Acetonitrile/Deionized water
Flow Rates	1.0 mL/min
Injection Volume	30 µL
Temperature (Column)	Ambient (room temperature)
Wavelength	290 nm for TDZ and photo-TDZ
Limit of Determination	0.05 ppm for cottonseed and 0.5 ppm for gin trash
Retention Time	7.8 min for TDZ and 11.1 min for photo-TDZ
Run Time	22 minutes

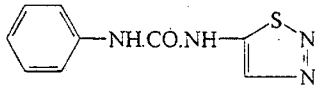
Table 2 Typical Recovery Data

Matrix	Compound		
	Fortification Level (ppm)	% Recovery Thidiazuron	% Recovery Photo-Thidiazuron
Cottonseed	0.05	97	107
Cottonseed	0.05	94	108
Cottonseed	0.05	71	108
Cottonseed	0.05	76	103

Matrix	Compound		
	Fortification Level (ppm)	% Recovery Thidiazuron	% Recovery Photo-Thidiazuron
Cottonseed	0.50	93	100
Cottonseed	0.50	72	103

Matrix	Compound		
	Fortification Level (ppm)	% Recovery Thidiazuron	% Recovery Photo-Thidiazuron
Gin trash	0.50	74	91
Gin trash	1.0	76	93
Gin trash	1.0	77	93
Gin trash	10.0	79	88

Figure 14 Structures



Thidiazuron

C.A. Name: N-phenyl-N'-1,2,3-thiadiazol-5-ylurea
IUPAC Name: 1-phenyl-3-(1,2,3-thiadiazol-5-yl)urea

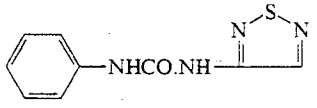


Photo-Thidiazuron

C.A. Name: N-phenyl-N'-1,2,5-thiadiazol-3-ylurea
IUPAC Name: 1-phenyl-3-(1,2,5-thiadiazol-3-yl)urea

Figure 15 Typical Calibration Data for Thidiazuron

Thidiazuron Standard Reference #	Thidiazuron Concentration (µg/mL)	Thidiazuron Peak Height Count	Calculated Line
AW/62/06	0.05	419	421
AW/62/03	0.10	852	844
AW/62/07	0.25	2130	2114
AW/62/05	0.50	4195	4229
AW/62/02	1.00	8471	8459

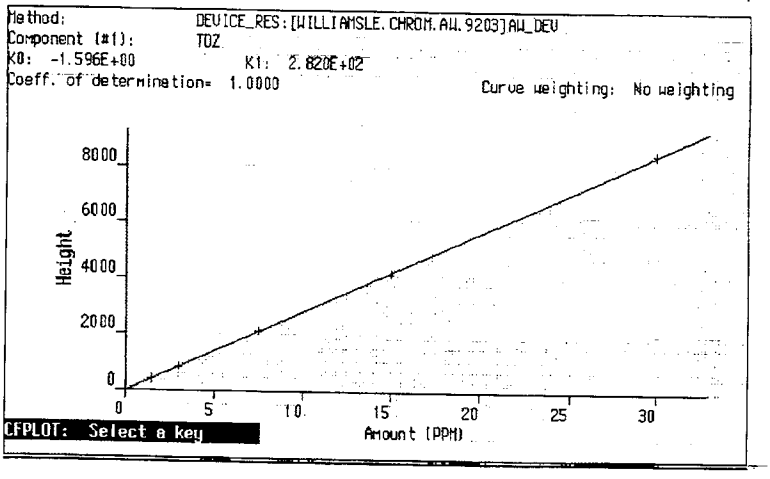


Figure 16 Typical Calibration Data for Photo-Thidiazuron

Photo-Thidiazuron Standard Reference #	Photo-Thidiazuron Concentration (µg/mL)	Photo-Thidiazuron Peak Height Count	Calculated Line
AW/62/06	0.05	266	250
AW/62/03	0.10	487	494
AW/62/07	0.25	1207	1226
AW/62/05	0.50	2455	2446
AW/62/02	1.00	4885	4885

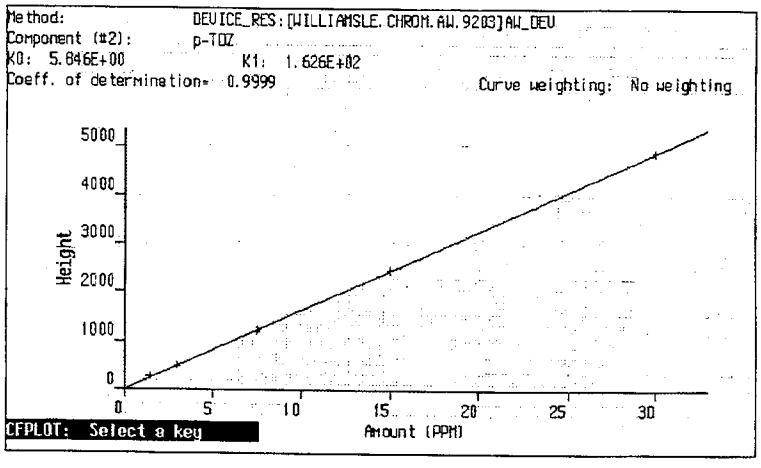
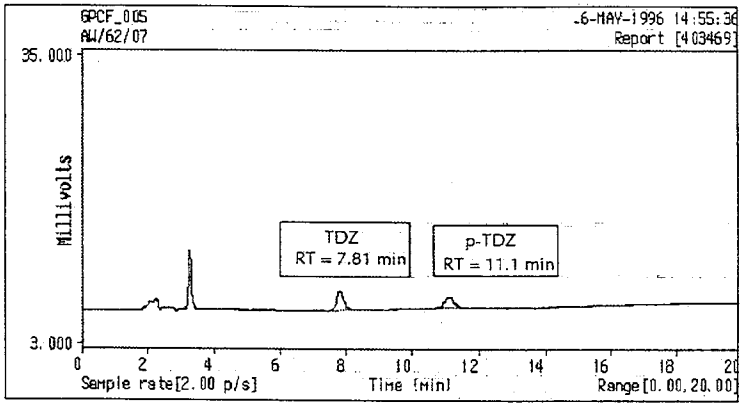


Figure 17 Typical Chromatograms for Standards and Various Matrices

0.25 µg/mL thidiazuron and photo-thidiazuron calibration standard



0.50 µg/mL thidiazuron and photo-thidiazuron calibration standard

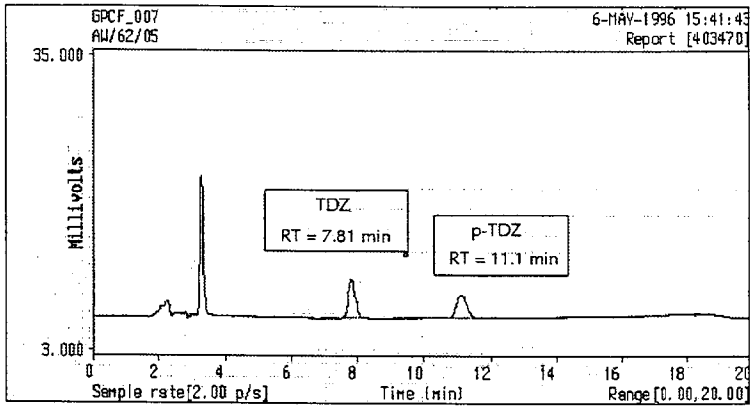
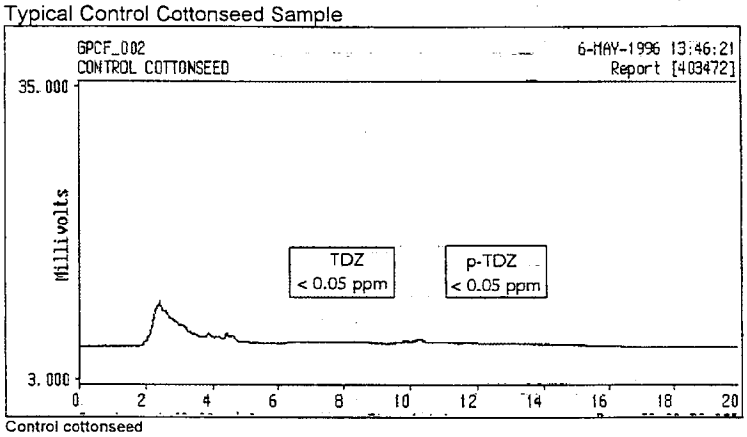


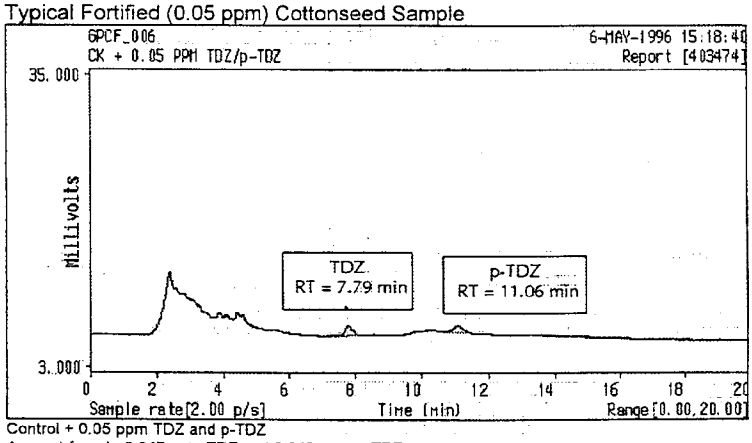
Figure 18

Typical Control Cottonseed Sample



Control cottonseed
Amount found: < 0.05 ppm TDZ and < 0.05 ppm p-TDZ
Amount added: NA

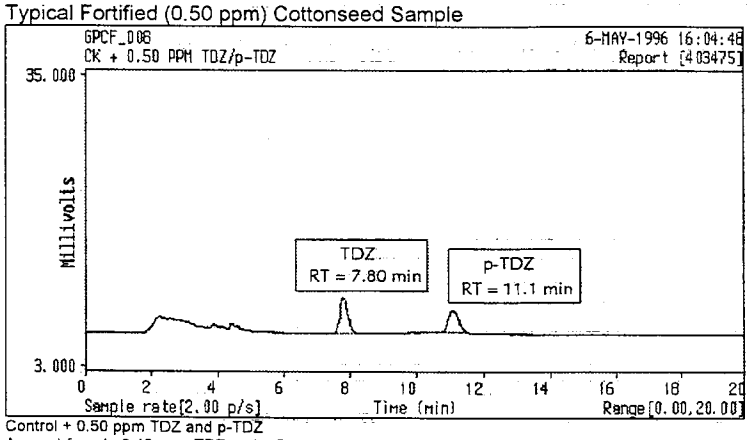
Typical Fortified (0.05 ppm) Cottonseed Sample



Control + 0.05 ppm TDZ and p-TDZ
Amount found: 0.047 ppm TDZ and 0.042 ppm p-TDZ
Amount added: 0.05 ppm TDZ and p-TDZ, respectively
Percent recovery: 94 for TDZ and 108 for p-TDZ

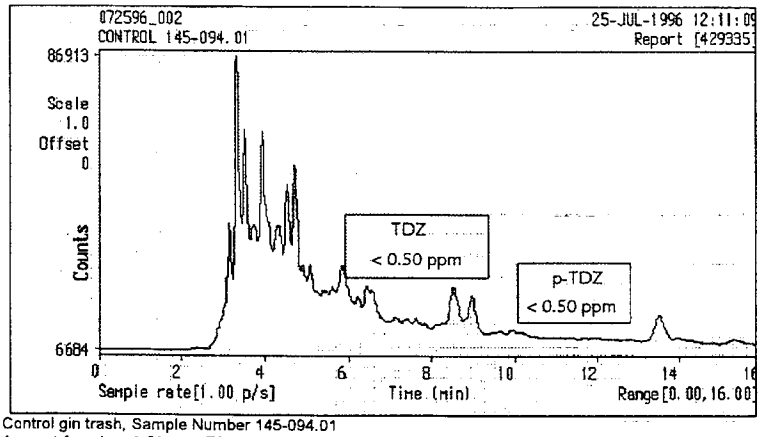
Figure 19

Typical Fortified (0.50 ppm) Cottonseed Sample



Control + 0.50 ppm TDZ and p-TDZ
Amount found: 0.46 ppm TDZ and 0.50 ppm p-TDZ
Amount added: 0.50 ppm TDZ and p-TDZ, respectively
Percent recovery: 93 for TDZ and 100 for p-TDZ

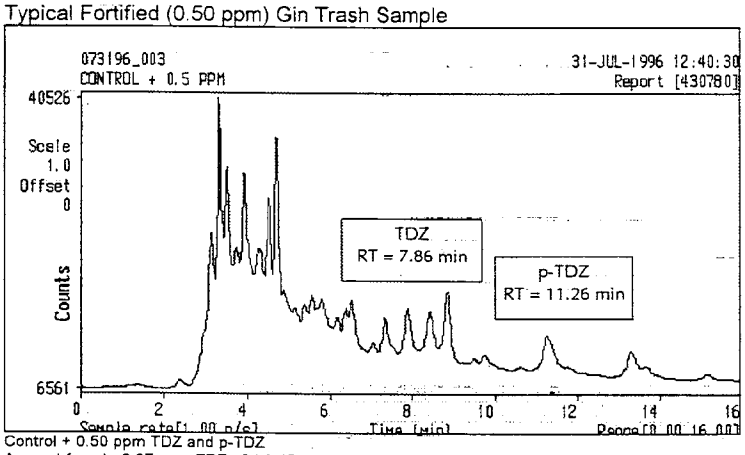
Typical Control Gin Trash Sample



Control gin trash, Sample Number 145-094.01
Amount found: < 0.50 ppm TDZ and < 0.50 ppm p-TDZ
Amount added: NA

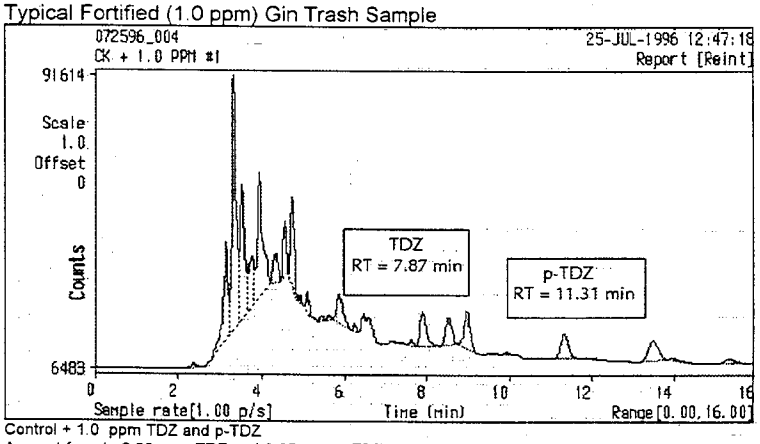
Figure 20

Typical Fortified (0.50 ppm) Gin Trash Sample



Control + 0.50 ppm TDZ and p-TDZ
Amount found: 0.37 ppm TDZ and 0.45 ppm p-TDZ
Amount added: 0.50 ppm TDZ and p-TDZ, respectively
Percent recovery: 75 for TDZ and 91 for p-TDZ

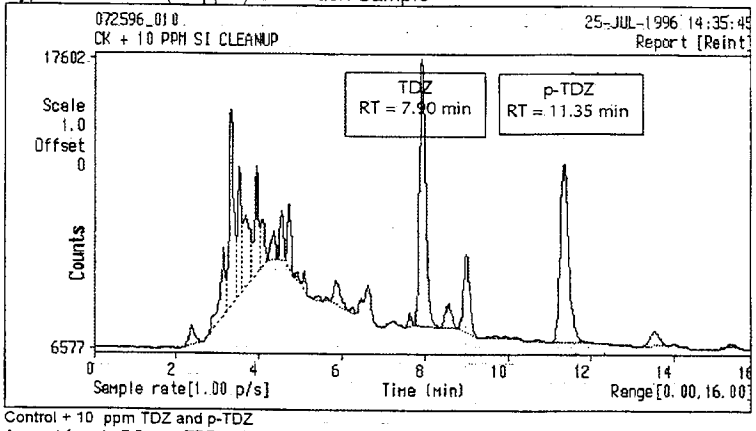
Typical Fortified (1.0 ppm) Gin Trash Sample



Control + 1.0 ppm TDZ and p-TDZ
Amount found: 0.80 ppm TDZ and 0.95 ppm p-TDZ
Amount added: 1.0 ppm TDZ and p-TDZ, respectively
Percent recovery: 80 for TDZ and 95 for p-TDZ

Figure 21

Typical Fortified (10 ppm) Gin Trash Sample



Control + 10 ppm TDZ and p-TDZ
Amount found: 7.9 ppm TDZ and 8.8 ppm p-TDZ
Amount added: 10 ppm TDZ and p-TDZ, respectively
Percent recovery: 79 for TDZ and 88 for p-TDZ

Figure 22 Flow Diagram of Cottonseed Method

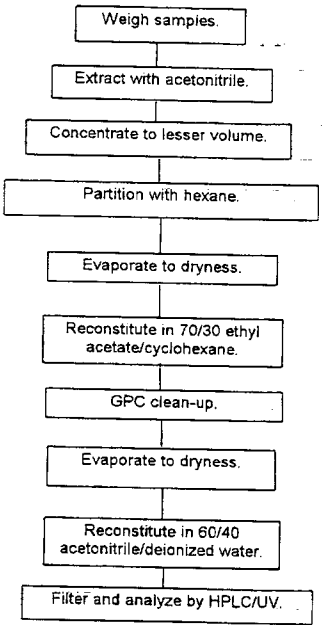
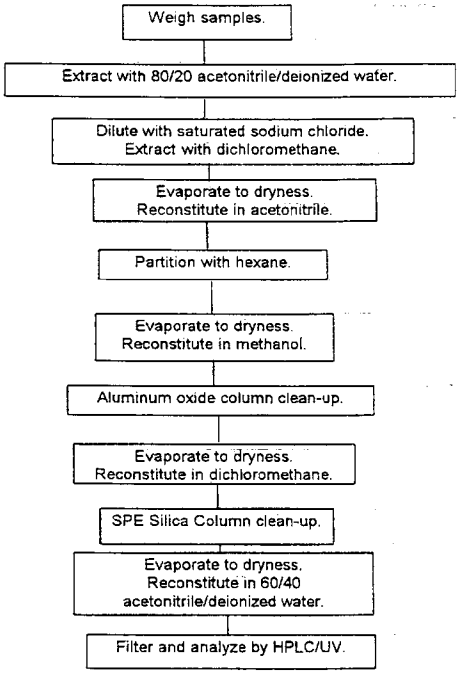


Figure 23 Flow Diagram of Gin Trash Method



III. Analysis of cottonseed and gin trash by the revised method (AW/02/96)

Cottonseed and gin trash were analyzed. In addition to the samples from AW-94R-08, samples from a metabolism study conducted at AgrEvo Research Center were also analyzed for the purpose of radio-validating the method. (See Section IV).

Recoveries and apparent residues in control samples are presented in Tables 3 and 4.

Table 3 Thidiazuron Residue Results Found in Recovery and Control Samples

State	Trial No.	Matrix	Sample ID	Amount Found	Fortification Level	% Found
CA	WFRS	Cottonseed	135-180.01	ND	–	–
		Cottonseed	135-180.01 + 0.05 ppm	0.0459	0.05	92
		Cottonseed	135-180.01 + 0.10 ppm	0.0952	0.10	95
		Gin trash	135-042.02	0.023	–	–
		Gin trash	135-042.02 + 0.50 ppm	0.3706	0. 50	74
		Gin trash	135-042.02 + 1.0 ppm	0.7898	1.0	79
		Gin trash	135-042.02 + 10 ppm	8.5759	10	86
NC [a]		Cottonseed	677E	0.01, ND	–, –	–
		Cottonseed	678E	ND, ND	–, –	–
		Cottonseed [b]	677E + 0.05 ppm	0.0513	0.05	103
		Cottonseed [a]	678E + 0.10 ppm	0.103	0.10	103

[a] Samples used for radio-validation.
[b] The average of two injections.

Table 4 Photo Thidiazuron Residue Results Found in Recovery and Control Samples

State	Trial No.	Matrix	Sample ID	Amount Found	Fortification Level	% Found
CA	WFRS	Cottonseed	135-180.01	ND	–	–
		Cottonseed	135-180.01 + 0.05 ppm	0.0479	0.05	96
		Cottonseed	135-180.01 + 0.10 ppm	0.1090	0.10	109
		Gin trash	135-042.02	0.034	–	<0.05
		Gin trash	135-042.02 + 0.50 ppm	0.4522	0. 50	91
		Gin trash	135-042.02 + 1.0 ppm	0.9100	1.0	91
		Gin trash	135-042.02 + 10 ppm	9.8735	10	99
NC [a]		Cottonseed	677E	ND, ND	–, –	–
		Cottonseed	678E	ND, 0.025	–, –	–
		Cottonseed [b]	677E + 0.05 ppm	0.0535	0.05	107
		Cottonseed [b]	678E + 0.10 ppm	0.086	0.10	86

[a] Samples used for radio-validation.
[b] The average of two injections.

In the case of control cottonseed, no peaks were detected at the retention time of thidiazuron or photo-thidiazuron. In the case of control gin trash however, peaks co-eluting with both thidiazuron and photo-thidiazuron were observed and these produced apparent residues of 0.023 ppm and 0.034 ppm for thidiazuron and photo-thidiazuron respectively.

Averaged over all fortified samples analyzed in the course of this work, the recovery of thidiazuron was 90.3% ± 11.3% (n = 7) and the recovery of photo-thidiazuron was 97.0% ± 8.6% (n = 7).

Residues in treated samples are presented in Tables 5 and 6. Figures 24 - 28 show typical chromatograms.

Table 5 Thidiazuron Residues Found in Treated Cottonseed and Gin Trash Using the Revised Method

State	Trial No.	Matrix	Sample ID	Residue (ppm)	Mean ± SD
CA	WFRS	Cottonseed	134-180.02 A	0.17	
		Cottonseed	134-180.02 B	0.18	0.17 ± 0.026
		Cottonseed	134-180.02 C	0.15	
		Gin Trash	135-042.10 A	18	
		Gin Trash	135-042.10 B	18	19 ± 1.6
		Gin Trash	135-042.10 C	21	

Cottonseed residues are corrected for a recovery of 94%.
Gin trash residues are corrected for a recovery of 79.6%.

Table 6 Photo Thidiazuron Residues Found in Treated Cottonseed and Gin trash Using the Revised Method

State	Trial No.	Matrix	Sample ID	Residue (ppm)	Mean ± SD
CA	WFRS	Cottonseed	134-180.02 A	< 0.05	
		Cottonseed	134-180.02 B	< 0.05	<0.05
		Cottonseed	134-180.02 C	< 0.05	
		Gin Trash	135-042.10 A	2.29	2.41 ± 0.24
		Gin Trash	135-042.10 B	2.25	
		Gin Trash	135-042.10 C	2.68	

Gin trash residues are corrected for a recovery of 93.7%.

A comparison of the results obtained using the revised method (AW/02/96) with those obtained using the original method (AW/02/95) is presented in Table 7. No measurable residues of photo-thidiazuron were found by either method and the residues of thidiazuron were essentially identical.

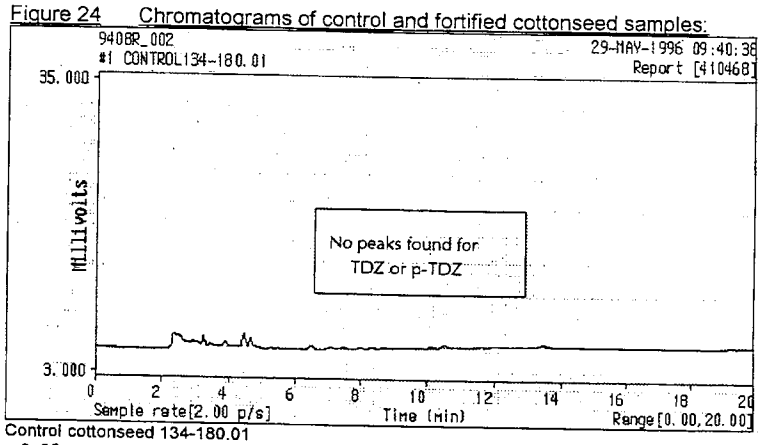
Table 7 A Comparison of Average Thidiazuron and p-Thidiazuron Residues in Treated Samples Analyzed by Method No. AW/02/95 and Method No. AW/02/96

State	Method	Matrix	TDZ (PPM)	p-TDZ(PPM)
CA	AW/02/95	Cottonseed	0.13 ± 0.012	<0.05
	AW/02/96	Cottonseed	0.17 ± 0.026	<0.05

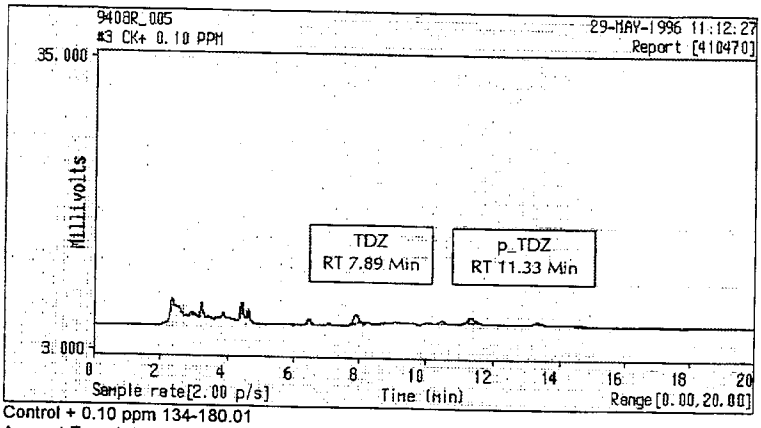
Each result is the mean of three analyses.

I. Typical chromatograms of cottonseed and gin trash.

Figure 24 Chromatograms of control and fortified cottonseed samples:

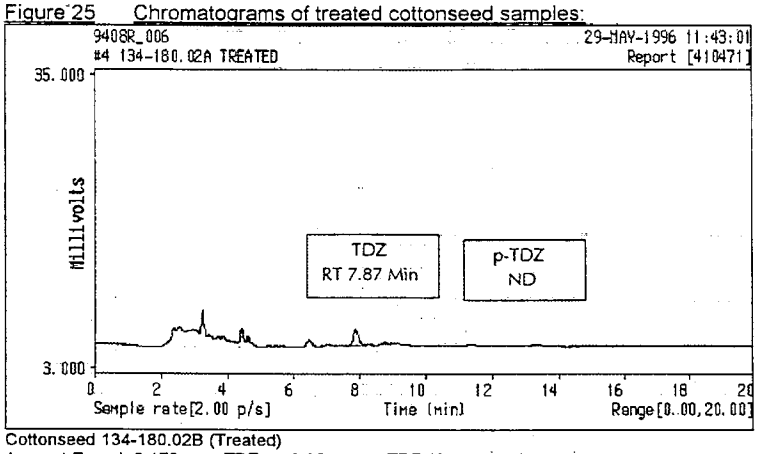


Control cottonseed 134-180.01
< 0. 05 ppm TDZ, < 0.05 ppm p-TDZ found.

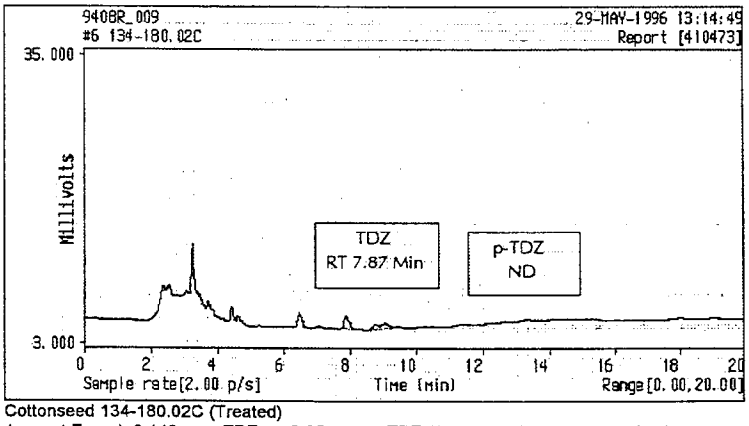


Control + 0.10 ppm 134-180.01
Amount Found: 0.0955 ppm TDZ, 0.1090 ppm p-TDZ
Amount Added: 0.10 ppm
Recovery: 96% TDZ and 109% p-TDZ

Figure 25 Chromatograms of treated cottonseed samples:

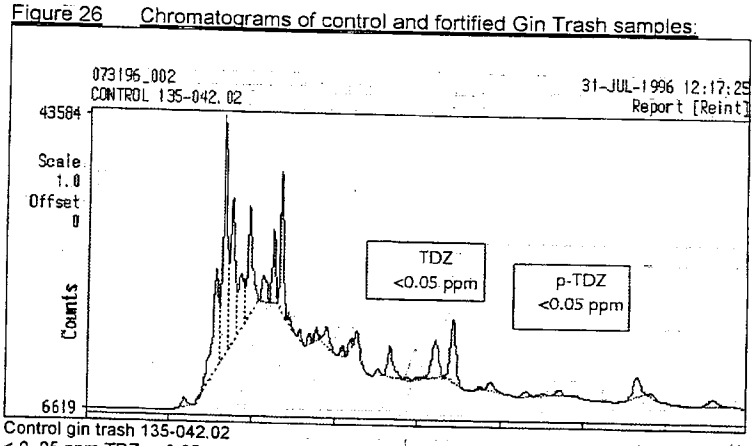


Cottonseed 134-180.02B (Treated)
Amount Found: 0.178 ppm TDZ, < 0.05 ppm p-TDZ (Corrected for recovery value.)

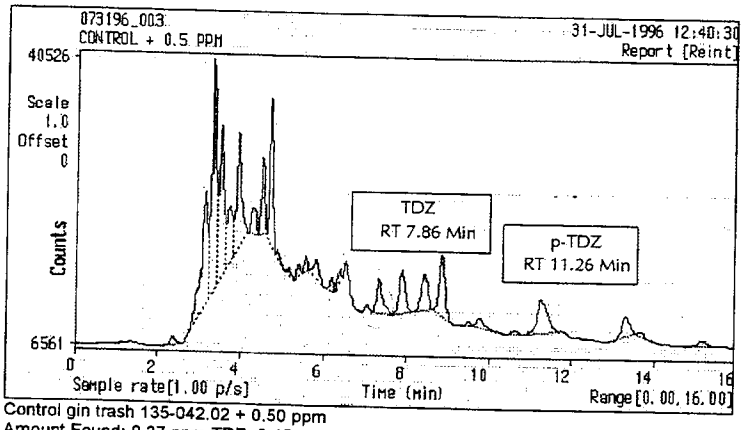


Cottonseed 134-180.02C (Treated)
Amount Found: 0.148 ppm TDZ, < 0.05 ppm p-TDZ (Corrected for recovery value.)

Figure 26 Chromatograms of control and fortified Gin Trash samples:

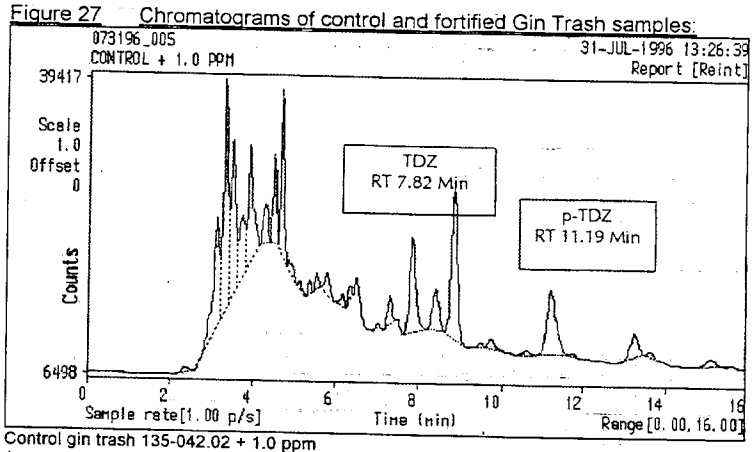


Control gin trash 135-042.02
< 0. 05 ppm TDZ, < 0.05 ppm p-TDZ found. (Corrected for Control results.)



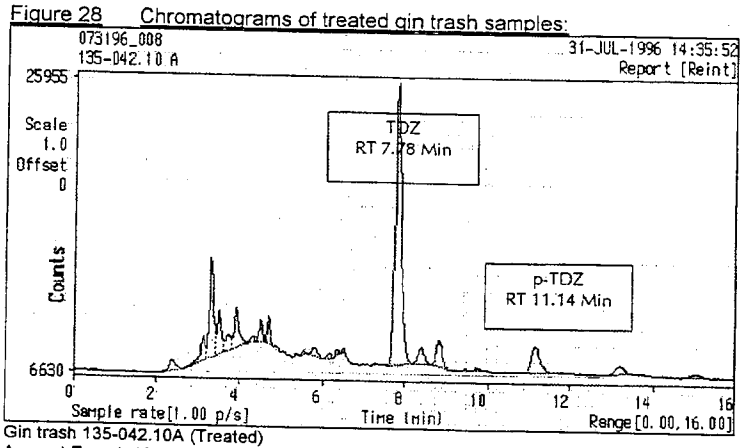
Control gin trash 135-042.02 + 0.50 ppm
Amount Found: 0.37 ppm TDZ, 0.45 ppm p-TDZ
Amount Added: 0.50 ppm
Recovery: 74% TDZ and 91% p-TDZ (Corrected for Control results.)

Figure 27 Chromatograms of control and fortified Gin Trash samples:

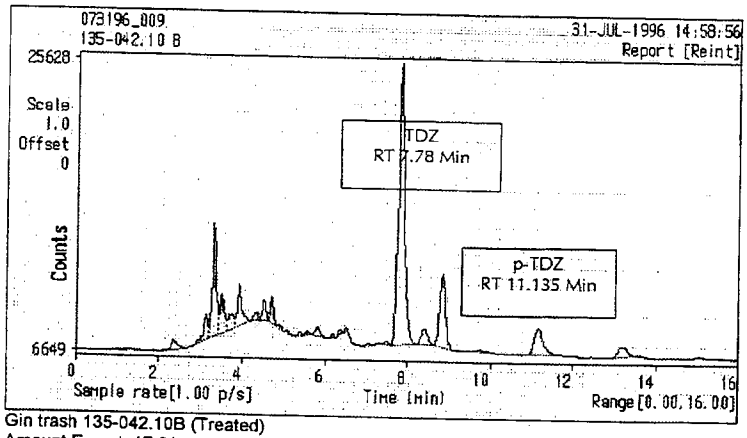


Control gin trash 135-042.02 + 1.0 ppm
Amount Found: 0.790 ppm TDZ, 0.91 ppm p-TDZ
Amount Added: 1.0 ppm
Recovery: 79% TDZ and 91% p-TDZ (Corrected for Control results.)

Figure 28 Chromatograms of treated gin trash samples:



Gin trash 135-042.10A (Treated)
Amount Found: 18.08 ppm TDZ, 2.29 ppm p-TDZ



Gin trash 135-042.10B (Treated)
Amount Found: 17.61 ppm TDZ, 2.25 ppm p-TDZ

IV. Radiovalidation

Samples of cottonseed containing field-incorporated residues of ^{14}C-thidiazuron were provided by Metabolism Department. These samples originated from study number 524AW, "Uptake of [5-^{14}C-thidiazol]-thidiazuron residues in soil by rotational crops under confined conditions". The objective of the study was to investigate the uptake of a soil metabolite of thidiazuron by crops rotated after cotton. Cotton plants growing in stainless steel tanks out-doors were treated with ^{14}C labeled thidiazuron formulated as Dropp 50WP. The cotton was at the correct stage of growth for defoliation and the nominal application rate was 0.3 lb ai/A, (46.7 mg of thidiazuron in 65.1 mL of spray solution applied to a tank with a surface area of 1.39 m^2). Cotton bolls were harvested and ginned by hand. This study is still in progress and will be reported when completed.

The ginned cottonseed was prepared for analysis as described in the revised method AW/02/96. Replicate aliquots of the finely ground seed were combusted for determination of total ^{14}C residue in the seed. Aliquots from the same samples were analyzed by the revised method AW/02/96.

Accountability was over 75% in each of the three samples. No measurable residues of photo-thidiazuron were present. Chromatograms are shown in Figures 29 - 30. These results indicate that method AW/02/96 accounts for almost three quarters of the total thidiazuron-derived residue in cottonseed from plants treated at normal field rate. Radiovalidation of the method has, therefore, been achieved.

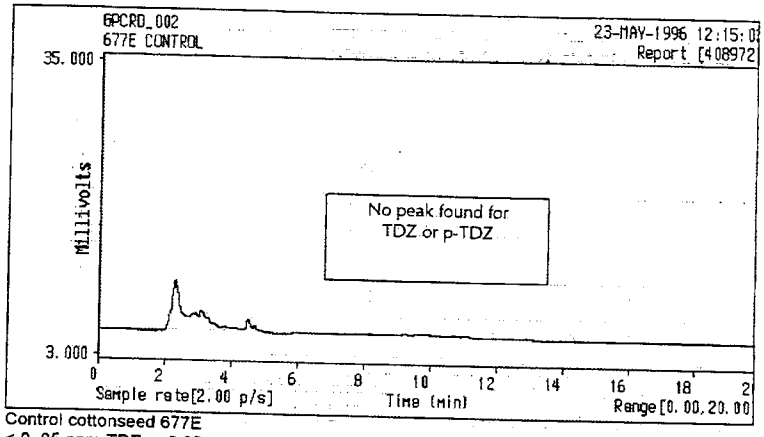
Results are presented in Table 8.

Table 8 Accountability of Field-incorporated Residues of ^{14}C-Thidiazuron in Cottonseed Analyzed by Method # AW/02/96

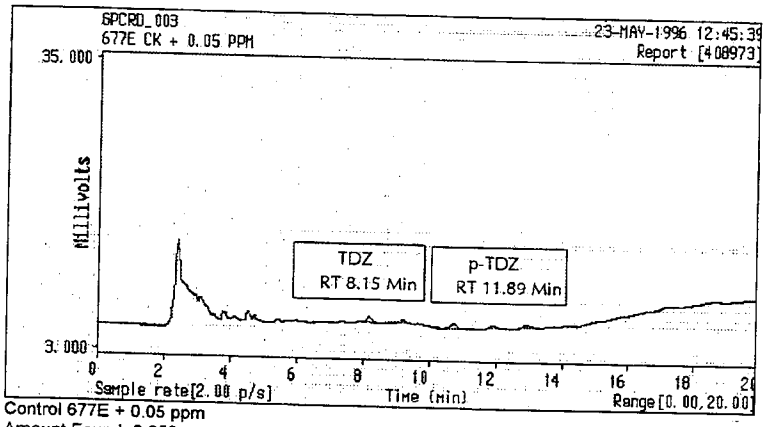
Sample #	Total ^{14}C Residue [a]	Thidiazuron [b]	% Accountability
679E	0.059	0.046	78
680E	0.120	0.094	78
681E	0.062	0.055	89

All results are expressed as ppm.
[a] Expressed as ppm thidiazuron
[b] Results corrected for a recovery of 90.4%

Figure 29 Chromatograms of Radiolabel ^{14}C treated cottonseed samples:



Control cottonseed 677E
< 0. 05 ppm TDZ, < 0.05 ppm p-TDZ found.



Control 677E + 0.05 ppm
Amount Found: 0.058 ppm TDZ, 0.0505 ppm p-TDZ
Amount Added: 0.05 ppm
Recovery: 116% TDZ and 101% p-TDZ

Appendix I Data Calculation Sheets

Cottonseed

Sample Number	Peak Area	Sample Weight (gm)	TDZ Found (µg/mL)	Crop Solvent Ratio	Compound Found (PPM)	Fort. Level (PPM)	Net % Recovery
135-180.01CK	N/D	20.25	N/D	2.5313	N/D		
135-180.01 + 0.05	460	20.10	0.115	2.5125	0.0459	0.05	92
135-180.01 + 0.10	1003	20.20	0.240	2.5250	0.0952	0.10	96
135-180.02 A	1733	20.06	0.408	2.5075	0.1629		
135-180.02 B	1780	20.02	0.419	2.5025	0.1675		
135-180.02 C	1473	20.02	0.349	2.5025	0.1393		

ND = Not Detected

Sample Number	Peak Area	Sample Weight (gm)	PTDZ Found (µg/mL)	Crop Solvent Ratio	Compound Found (PPM)	Fort. Level (PPM)	Net % Recovery
135-180.01CK	N/D	20.25	N/D	2.5313	N/D		
135-180.01 + 0.05	237	20.10	0.120	2.5125	0.0479	0.05	96
135-180.01 + 0.10	626	20.20	0.275	2.5250	0.1090	0.10	109
135-180.02 A	30	20.06	0.038	2.5075	<0.05		
135-180.02 B	ND	20.02	<0.05	2.5025	<0.05		
135-180.02 C	ND	20.02	<0.05	2.5025	<0.05		

ND = Not Detected

Appendix I (continued) Data Calculation Sheets

Radiolabel Cottonseed

Sample Number	Peak Area	Sample Weight (gm)	TDZ Found (µg/mL)	Crop Solvent Ratio	Compound Found (PPM)	Fort. Level (PPM)	Net % Recovery
677E	80	20.00	0.028	2.500	<0.050		
678E	ND	20.00	<0.05	2.500	<0.050		
677E + 0.05	458*	20.00	0.112	2.500	0.0448	0.05	90
678E + 0.10	1024	20.03	0.234	2.504	0.0936	0.10	94
679E	439	20.07	0.108	2.509	0.0430		
680E	**	20.03					
681E	603	20.04	0.144	2.505	0.0576		

* 530 - 80 = 458
** Sample lost on injection
ND = Not Detected

Sample Number	Peak Area	Sample Weight (gm)	PTDZ Found (µg/mL)	Crop Solvent Ratio	Compound Found (PPM)	Fort. Level (PPM)	Net % Recovery
677E	ND	20.00	<0.05	2.500	<0.050		
678E	41	20.00	0.062	2.500	0.062		
677E + 0.05	250	20.00	0.141	2.500	0.0448	0.05	113
678E + 0.10	404*	20.03	0.200	2.504	0.0799	0.10	80
679E	ND	20.07	<0.05	2.509	<0.05		
680E	ND	20.03	<0.05	2.504	<0.05		
681E	ND	20.04	<0.05	2.505	<0.05		

* 445 - 41 = 404
ND = Not Detected

Appendix I (continued) Data Calculation Sheets

Radiolabel Cottonseed (continued)

Sample Number	Peak Area	Sample Weight (gm)	TDZ Found (µg/mL)	Crop Solvent Ratio	Compound Found (PPM)	Fort. Level (PPM)	Net % Recovery
677E	ND	20.00	<0.05	2.500	<0.050		
678E	ND	20.00	<0.05	2.500	<0.050		
677E + 0.05	605	20.00	0.145	2.500	0.0578	0.05	116
678E + 0.10	1147	20.03	0.276	2.504	0.1103	0.10	110
679E	466	20.07	0.111	2.509	0.0442		
680E	882	20.03	0.212	2.504	0.0846		
681E	448	20.04	0.106	2.505	0.0425		

ND = Not Detected

Sample Number	Peak Area	Sample Weight (gm)	PTDZ Found (µg/mL)	Crop Solvent Ratio	Compound Found (PPM)	Fort. Level (PPM)	Net % Recovery
677E	ND	20.00	<0.05	2.500	<0.050		
678E	ND	20.00	<0.05	2.500	<0.05		
677E + 0.05	223	20.00	0.126	2.500	0.0505	0.05	101
678E + 0.10	476	20.03	0.231	2.504	0.0922	0.10	92
679E	ND	20.07	<0.05	2.509	<0.05		
680E	ND	20.03	<0.05	2.504	<0.05		
681E	ND	20.04	<0.05	2.505	<0.05		

ND = Not Detected