4.5 Method of Analysis

Sporadic occurrences of trace levels of interferences were observed during the study. These interferences were separated from the analytes by fine-tuning the chromatographic parameters. The chromatographic parameters and a synopsis of the analytical methods used are listed in Section 4.5.1-4.5.4. Detailed method descriptions may be found in Appendix V.

NOTE: Most of the chromatography was performed using two 10-meter wide-bore HP-1 columns. Despite using columns of the same type, a large difference in retention characteristics was noted between the two columns. This discrepancy caused an anomaly in the retention time listings in Tables 5, 6, and 7 where the retention times seem to shorten as the column oven temperature is cooled. Differentiation between the two columns was not noted in the raw data.

4.5.1 Amitraz

The method used for the analysis of amitraz in soil has been reported earlier[8].

Amitraz was extracted from the moist soil by shaking it vigorously with acetone. The extract was filtered and taken to dryness (or near dryness) using a rotary evaporator. If the samples contained enough moisture such that dryness was not readily achieved through rotary evaporation, the remaining water was quantitatively transferred to a vial, extracted with dichloromethane and the extract dried under a gentle stream of air.

Once the sample was in a dry form (either through rotary evaporation or extraction), the residues were transferred to a volumetric flask using ethyl acetate followed by toluene.

Quantification was achieved using a gas chromatograph equipped with a nitrogen-phosphorous detector. Due to varying interferences detected throughout the study, the chromatographic conditions were modified as the need arose. These conditions are described below.

Gas Chromatographic Conditions:

Instrument (two models used):

(a) Hewlett-Packard HP5890A gas chromatograph equipped with a nitrogen-phosphorous detector (NPD).
(b) Hewlett-Packard HP5880A gas chromatograph equipped with NPD.

Column: see Table 5

Temperature: Injector = 250°C
Oven = see Table 5
Detector = 280°C

Gases: Carrier = helium at 30 ml/minute
Septum purge = helium at 3 ml/minute
Split flow = helium at 20 ml/minute
Detector air = 110 ml/minute
Detector hydrogen = 3.5 ml/minute

Table 5

Retention Time Data for Amitraz

Column	Oven Temp. (°C)	Retention Time (min.)
HP-1, 10 meter x 0.54 mm (i.d.), 2.65 µm film	225 195 215	4.9-5.0 4.1 5.0-5.4
DB-1, 15 meter x 0.54 mm (i.d.), 3.0 µm film	215	7.5

4.5.2 BTS 27271

The analytical method for BTS 27271 has been reported previously[9].

Soil was suspended in alkaline water and BTS 27271 is extracted by shaking with toluene. The extract was purified by extraction into dilute acid, basification, and back-extraction into toluene.

Quantitation was achieved using a gas chromatograph equipped with a nitrogen-phosphorous detector. Varying forms and levels of chromatographic interference were experienced during the study. This problem was overcome by using different combinations of column types and oven temperatures as stated below.

Chromatographic Conditions:

Instrument (two models used):

(a) Hewlett-Packard HP5890A gas chromatograph equipped with NPD.
(b) Hewlett-Packard HP5880A gas chromatograph equipped with NPD.

Column: see Table 6

Gases: Carrier = helium at 30-34 ml/minute
Split flow = helium at 18-20 ml/minute
Septum purge = helium at 3.0 ml/minute
Detector hydrogen = 3.0-5.8 ml/minute
Detector air = 91-110 ml/minute

Temperatures: Injector = 250°C
Oven = see Table 6 for details
Detector = 280 or 350°C

(Project begun at 280°C. Set-point was charged to 350°C to help keep NPD cleaner during operation. The change did not affect the chromatography.)

Table 6

Retention Time Data for BTS 27271

Column	Oven Temp. (°C)	Retention Time (min.)
HP-1, 10 meter x 0.54 mm (i.d.), 2.65 µm film	105 100 125	2.2-2.7 3.4-3.5 3.2-3.5
DB-1, 15 meter x 0.54 mm (i.d.), 3.0 µm film	130 140	4.2-5.0 3.3-3.5

NOTE: In a few of the sample runs, a late-eluting interference was noted which was removed by rapidly increasing the temperature of the oven to 200°C and holding for about 8 minutes.

4.5.3 BTS 27919

The analytical method used for quantifying BTS 27919 in soil has been previously reported.[10]

BTS 27919 was extracted by percolating distilled toluene through the soil using a soxhlet extractor. The volume of the extract was reduced using a rotary evaporator, and the BTS 27919 purified using a silica gel cartridge. The volume was adjusted prior to chromatography using a nitrogen-phosphorous detector to quantify the residues.

Varying forms and levels of chromatographic interferences were experienced throughout the study. These problems were successfully overcome using several different column types in combination with variation of the oven temperatures as described below.

Chromatographic Conditions

Instrument (two models used):

(a) Hewlett-Packard HP5890A gas chromatograph equipped with NPD.
(b) Hewlett-Packard HP5880A gas chromatograph equipped with NPD.

Column: see Table 7

Gases: Carrier = helium at 30 ml/minute
Split flow = helium at 20 ml/minute
Septum purge = helium at 3.0 ml/minute
Detector hydrogen = 3.5 ml/minute
Detector air = 110 ml/minute

Temperature: Injector = 250°C
Detector = 280°C
Oven = (see Table 7)

Table 7

Retention Time Data for BTS 27919

Column	Oven Temp. (°C)	Retention Time (min.)
HP-1, 10 meter, 0.54 mm (i.d.), 2.65 µm film	90 95 100 115 125	3.5-3.6 7.9 2.1-2.2 4.1 2.3-2.5
DB-1, 15 meter, 0.54 mm (i.d.), 3.0 µm film	100 130	7.0-7.5 3.1

NOTE: In a few of the sample runs, a late-eluting interference was noted which was removed by rapidly increasing the temperature of the oven to 200°C and holding for about 8 minutes.

4.5.4 Total Residues

A method which hydrolyzes amitraz-related residues to a common moiety was used as a confirmatory analysis. This method has been previously reported[11] and is summarized below. A detailed description appears in Appendix V.

Amitraz and its metabolites are hydrolyzed to 2,4-dimethylaniline (DMA) by boiling the soil with aqueous base. The DMA is concurrently steam-distilled and trapped in hexane. The extract is purified by extraction into dilute acid followed by basification and back-extraction into hexane. The DMA is derivatized with heptafluorobutyric anhydride, and the product is purified through silica gel.

Quantitation is achieved by using a gas chromatograph equipped with an electron-capture detector.

Gas Chromatographic Conditions

Instrument: Hewlett-Packard HP5790 gas chromatograph equipped with a Ni-63 electron-capture detector.

Column: DB-17 (J&W), 30 meter x 0.25 mm
(i.d.), 0.25 µm film

Gases: Carrier = helium at 13 psig
(linear vel. = 20 cm/sec)
Split Flow = helium at 18 ml/minute
Septum Purge = helium at 3 ml/minute
Make-up = nitrogen at 45 ml/minute

Temperature: Injector = 250°C
Oven = 145°C
Detector = 350°C

Retention time of derivatized DMA= 5.3 minutes

5.1 Method Efficiencies

The recovery data from all three methods were acceptable. Difficulty with the chromatography of BTS 27271 (moderate tailing of the analyte) led to broader scattering of recoveries. At levels of 0.02 and below, recoveries in excess of 100% were often recorded. The main contributor to these high recoveries was a slight y-intercept of the best-fit straight line. This effect was negligible above 0.02 ppm. Although accurate quantitation of residues below 0.02 ppm was not possible, it was clear that the analytical methods were sensitive to this level and, if anything, overestimated very low residues. Residues below twice the limit of determination of 0.01 ppm were not used in calculating half-lives.