Appendix III (continued)

A. Analysis of Sediment and Soil Samples

1. A small amount (50 g) of well-homogenized sample was placed in a polyethylene screw-cap bottle. Fortification of the sample with flutolanil and desisopropyl flutolanil (DIP) dissolved in 90% hexane/10% acetone (v/v) was performed at this point. NOTE: The sample size was reduced to 20 g in some instances due to the high organic content.

2. A 10% water/90% acetone (v/v) (250 ml) mixture was added to the bottle which was sealed and shaken for thirty minutes.

3. The extract was filtered through a glass-fiber filter and remaining solids were washed with acetone (100 ml). The resulting filtrate was transferred to a boiling flash and the acetone was removed using a rotary evaporator (bath at 40°C).

4. The resulting aqueous extract was transferred to a separatory funnel with two washes of a 5% sodium chloride (w/v) solution (25 ml each).

5. Flutolanil was extracted from this mixture with two portions (50 ml each) of hexane. DIP was then extracted with two portions (50 ml each) of dichloromethane. The extract for each compound was kept separate and each was filtered through anhydrous sodium sulfate to remove any traces of moisture and taken to dryness using a rotary evaporator (bath at 40°C).

6. Flutolanil was purified through a column containing Florisil (10 g) which had been partially deactivated by adding enough water to equal 1% of its final weight and capped with anhydrous sodium sulfate. The column was rinsed with 10% ethyl acetate/90% hexane (v/v) (80 ml). The residues of flutolanil were then applied to the columns as three rinses (5 ml each) of the boiling flash. The column was then washed with 10% ethyl acetate/90% hexane (v/v) (80 ml) and this wash was discarded. Flutolanil was then eluted with 20% ethyl acetate/80% hexane (v/v) (100 ml). The column eluent was then taken to dryness using a rotary evaporator (bath at 40°C) and reconstituted in 10% acetone/90% hexane (v/v) (2.0 ml) for gas chromatography (see Sec. D).

Desisopropyl flutolanil was derivatized to a dimethyl analog by transferring the residues from the boiling flask to a 25 x 50 mm glass vial with three portions (2 ml each) of acetone. The acetone was then dispelled and the residues were dissolved in dimethyl sulfoxide (1.6 ml). Methyl iodide (0.4 ml) and 5N sodium hydroxide (0.2 ml) were added to the vial which was then sealed and incubated at 40°C for two hours. The vial was then cooled and the reaction was stopped by adding water (10 ml). The dimethyl analog (dimethyl DIP) was then extracted from the mixture with three portions (5 ml each) of hexane. The hexane extract was taken to dryness under a stream of nitrogen or air, reconstituted in hexane (2.0 ml) and analyzed using a gas chromatograph (see Sec. D).

B. Characterization of Sediment and Soil Samples

1. Density (sediment only)

a. The density of each sediment sample analyzed was determined by weighing a small amount of sediment (about 15 g) into a pre-weighed graduated centrifuge tube and recording its weight.

b. The sample was then placed into a centrifuge and spun until it settled to the bottom of the tube. The value of the sample was then recorded. The density of the sediment was then calculated using equation 1.

$$\text{density, g/ml} = \frac{\text{wt. sed. + tube, g - wt. tube, g}}{\text{vol. sample, ml}} \qquad \text{(Eq. 1)}$$

2. Gravimetric moisture content (GMC)

a. A subsample (ca. 10 grams) of sediment or soil was placed onto a pre-weighed aluminum boat and weighed.

b. The sample was then placed in an oven (100-110°C) overnight.

c. The sample was then weighed and the GMC was determined using equation 2.

$$GCM = \frac{(wt\ wet\ sample + boat,\ g) - (wt\ dry\ sample + boat,\ g)}{(wt\ dry\ sample + boat,\ g) - (wt\ boat,\ g)}$$

(Eq. 2)

C. Analysis of Water Samples

1. A small amount of the well-mixed water sample (50 ml) was poured into a separatory funnel and 5% sodium chloride solution (w/v) (50 ml) was added to it.

2. Flutolanil was extracted from the sample using two portions (50 ml each) of hexane. DIP was then extracted using two portions (50 ml each) of dichloromethane. The two extracts were kept separate and were both dehydrated by filtering through a pad of anhydrous sodium sulfate. The hexane extract was concentrated to approximately 5 ml using rotary evaporation (bath at 40°C), however the DIP extract was allowed to concentrate to dryness.

3. Flutolanil was purified through a column containing Florisil (10 g) which had been partially deactivated by adding enough water to equal 1% of its final weight and capped with anhydrous sodium sulfate. The column was rinsed with 10% ethyl acetate/90% hexane (v/v) (80 ml). The flutolanil extract was applied to the top of the column followed by two hexane rinses (5 ml each) of the boiling flask. The column was then washed with 10% ethyl acetate/90% hexane (v/v) (80 ml) and this wash was discarded. Flutolanil was then eluted with 20% ethyl acetate/80% hexane (v/v) (100 ml). The column eluent was then taken to dryness using a rotary evaporator (bath at 40°C) and reconstituted in 10% acetone/90% hexane (v/v) (2.0 ml) for gas chromatography (see Sec. D).

4. Desisopropyl flutolanil was derivatized to a dimethyl analog by transferring the residues from the boiling flask to a 25 x 50 mm glass vial with three portions (2 ml each) of acetone. The acetone was then dispelled and the residues were dissolved in dimethyl sulfoxide (1.6 ml). Methyl iodide (0.4 ml) and 5N sodium hydroxide (0.2 ml) were added to the vial which was then sealed and incubated at 40°C for two hours.

The vial was then cooled and the reaction was stopped by adding water (10 ml). The dimethyl analog (dimethyl DIP) was then extracted from the mixture with three portions (5-ml each of hexane. The hexane extract was taken to dryness under a stream of nitrogen or air, reconstituted in hexane (2.0 ml) and analyzed using a gas chromatograph (see Sec. D).

D. Gas Chromatography

Several gas chromatographic configurations were used to quantify the analytical targets in the sample extract. Table 1 lists the important details for each system used. Systems A, B, and C were employed in the analyses performed at ABC Labs and systems D and E were employed at the NRC.

Table AIII-1 Gas Chromatographic Conditions

	Chromatographic system used				
	A	B	C	D	E
Laboratory	ABC Labs	ABC Labs	ABC Labs	NRC	NRC
Instrument					
manufacturer	Hewlett-Packard	Hewlett-Packard	Hewlett-Packard	Hewlett-Packard	Hewlett-Packard
model	HP 5890	HP 5890	HP 5890	HP 5890	HP 5890
detector	NPD (a)	NPD	NPD	NPD	NPD
Column					
manufacturer	Hewlett-Packard	J&W	J&W	Hewlett-Packard	Hewlett-Packard
stationary phase	HP-1	DB-1	DB-1	HP-1	HP-1
length (m)	5	12	15	5.0	5.0
inner dia. (cm)	0.53	0.53	0.53	0.53	0.53
film thickness. (um)	2.65	1.5	1.5	2.65	3.0
Temperatures					
injector	195 - 205 °C	195 - 205 °C	195 - 205 °C	250 °C	250 °C
column					
flutolanil	165 °C	180 °C	172 - 180 °C	165 - 168 °C (b)	190 °C (c)
dimethyl DIP	165 °C	155 °C	165 °C	165 - 168 °C (b)	190 °C (c)
detector	300 °C	300 °C	300 °C	350 °C	300 °C
Gas flows					
carrier (He)	25 ml/ min	29 ml/ min	25 - 32ml/ min	18 ml/ min	28-32 ml/ min
make-up (He)	1 - 5 ml/ min	1 - 5 ml/ min	1 - 5 ml/ min	none	none
air	100 ml/ min	100 ml/ min	100 ml/ min	80 ml/ min	98-103 ml/ min
hydrogen	4 ml/ min	4 ml/ min	4 ml/ min	4 ml/ min	3-4 ml/ min
Retention times					
flutolanil	4.7 min	3.9 min	3.9 - 10.3 min	4.7 - 4.8 min	4.8 - 5.3 min
dimethyl DIP	2.3 min	5.2 min	3.7 - 7.2 min	2.2 - 2.5 min	2.5 - 2.8 min
Calibration range (ug/ml)	0.05 to 5.0	0.05 to 5.0	0.05 to 5.0	0.4 to 1.2	0.16 to 1.0
Injection volume	4.0 ul	4.0 ul	4.0 ul	4.0 ul	3 - 4 ul (c)

(a) NPD = nitrogen-phosphorus detector.
(b) Column was heated to 240 - 250 °C after flutolanil had eluted.
(c) Held constant throughout the run.

E. Calculations

1. Linear regression analysis was performed on the data from the calibration standards (peak height vs. concentration) in order to obtain the best-fit line (see equation 3). Equation 4 was then derived from equation 3 and was used to calculate the concentration of the analytical analyte in each extract.

$$\text{peak height} = [(\text{slope}) \times (\text{conc. analyte*, } \mu g/ml)] + (\text{intercept}) \quad \text{(Eq. 3)}$$

$$\text{conc. analyte*, } \mu g/ml = [(\text{peak height}) - (\text{intercept})]/(\text{slope}) \quad \text{(Eq. 4)}$$

* flutolanil or dimethyl DIP

2. The uncorrected residues were then derived using equation 5.

$$\text{raw ppm} = \frac{(\text{conc. analyte, } \mu g/ml) \times (\text{vol. extract, ml}) \times MWF^*}{(\text{wt. sample analyzed, g})} \quad \text{(Eq. 5)}$$

* MWF = 1.00 for flutolanil or 0.91 for dimethyl DIP

3. The recovery value for the fortified samples was calculated by first calculating the target concentration of the compound in the sample using equation 6. Then any apparent residue in the control was subtracted from the uncorrected residue in the fortified sample before calculating the recovery as shown by equation 7. Recovery values were reported as percents (see equation 8).

$$\text{target conc., ppm} = \frac{(\text{conc. std. soln., } \mu g/ml) \times (\text{vol. added, ml})}{(\text{wt. sample fortified, g})} \qquad \text{(Eq. 6}$$

$$\text{recovery} = \frac{(\text{raw ppm of fort. sample}) - (\text{raw ppm of control})}{(\text{target conc., ppm})} \qquad \text{(Eq. 7}$$

$$\text{recovery, \%} = \text{recovery} \times 100\% \qquad \text{(Eq. 8}$$

4. Uncorrected residues were adjusted for the average recovery through the study and for sediment and soil, the GMC of the sample using equation 9.

$$\text{Corr. ppm} = \frac{(\text{raw ppm})}{(\text{avg. rec.})} \times (1 + GMC)^{*} \qquad \text{(Eq. 9}$$

* Soil and sediment only.