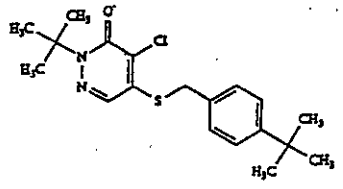
Analytical Method Trial at Huntingdon Analytical
Services for the Analysis of BAS 300 I in Pond Sediment

TEST SUBSTANCE

The following standard, received from BASF Corporation, Agricultural Research Center, P.O.Box 13528, 26 Davis Dr., Research Triangle Park, NC 27709-3528, was utilized for this study:

Product Name:	Pyridaben
Chemical Name:	2-tert-butyl-5-(4-tert-butylbenzylthio)-3(2H)-pyridazinone
CAS Number:	96489-71-3
Lot Number:	129S8604
Purity:	99.5%

Structure:



The analytical standard was stored at <-5°C when not in use. The standard calibration solutions were stored in a refrigerator (2°C-10°C) when not in use.

SUMMARY
BASF Analytical Method No. D9201 entitled "GC Method for Residue Determinations of Pyridaben (BAS 300 I) in Soil and Pond Sediment" (Reference 1) has been successfully validated in pond sediment at Huntingdon Analytical Services (HAS). The method trial was performed using control pond sediment received from Toxikon Environmental Sciences, Jupiter, FL 33477. The sediment samples were collected September 14, 1992 from Tank #7 (Reference 2).

INTRODUCTION
This report contains the validation data of Pyridaben as determined by HAS. The study was initiated October 2, 1992, the experimental starting date was October 14, 1992 and data were collected up to October 16, 1992. This validation adheres to the guidelines set forth in BASF Study Number 92162 (HAS Study Number A008.036).

The original final report, original raw data including laboratory notebooks, chromatograms with corresponding data, as well as an exact copy of remaining raw data will be retained in the BASF archives at 26 Davis Drive, Research Triangle Park, NC 27709. Copies of original data, protocol and final report will be retained in the HAS archives for 3 years, after which time, said data will be discarded. Facility raw data will be retained by HAS.

SAMPLE IDENTIFICATION
The pond sediment sample was received at Huntingdon on September 25, 1992 and stored at <-5°C (Room 120) until analysis. It was given a unique eight digit sample number, where:

1. 20 - Agrichemical Group
2. 594 - Batch number
3. Next three numbers - Individual sample number

HAS notebook/queue numbers were assigned as follows:

1. First three numbers: HAS notebook number
2. Next two numbers: notebook page number
3. Next two numbers: unique sample number in each analytical set
4. The last letter A,B,C, etc. was added for computer identification so that the actual sample ID (1+2+3) was not overwritten if the sample had to be re-injected.

For example: 4040603A (See Figure 7)
404 - HAS notebook number
06 - notebook page number
03 - unique sample number
A - injection for quantitation of Pyridaben

METHOD OF ANALYSIS

BASF Method D9201 was validated by fortifying duplicate control pond sediment samples with Pyridaben at 0.01, 0.02, 0.50 and 5.0 ppm. This was achieved by adding known amounts of Pyridaben standard solutions to the samples by Class A volumetric pipets. Duplicate control samples were also analyzed. The limit of quantitation was 0.01 ppm.

A brief description of the method follows. Pyridaben was extracted from pond sediment with methanol. The concentrated extract was acidified, and partitioned with dichloromethane (DCM). The residue was then subjected to Florisil column chromatography. Final quantitation was accomplished by capillary GLC using a ^{63}Ni electron capture detector. (See Figure 1)

QUANTITATION

Gas chromatographic conditions utilized at HAS for quantitation of pyridaben are listed below:

Hewlett Packard 5890 Gas Chromatograph

Column:	J&W DB-5; 30 meter; 0.32 mm i.d.; 1.0 μ film thickness
Column temperature:	Initial temperature was 250°C for 0.5 minute; ramp to 300°C at 15° per minute. Hold for 10 minutes; then ramp to 325°C at 15° per minute and hold for 10 minutes. Equilibration time was 3 minutes.
Detector temperature:	325°C
Injector temperature:	270°C
Gas Flows:	
Carrier:	
Helium	Head pressure set to 18 psi; total flow at 107 mL/minute
Detector:	
Argon:Methane (95:5)	60 mL/minute
Injection volume:	1 μL (splitless)

Gas chromatographic data were processed on a Perkin-Elmer computer using CLAS. The multi-level (linear regression) program was used to calculate the ppm found in control and fortified samples. Quattro Pro spreadsheets were used to summarize the data and to calculate recoveries from the fortified samples. Examples of the calculations used are shown in Figure 2.

REFERENCES

1. Nelson Delgado, "GC Method for the Determinations of Pyridaben (BAS 300 I) in Pond Sediment", BASF Method D9201, November 16, 1993, BASF Corporation, Research Triangle Park, NC 27709.
2. Gary M. Rand, Ph.D, Toxicon Environmental Sciences and Catherine M. Holmes, BASF Corporation, "Pyridaben (BAS 300 11 I): An Outdoor Aquatic Microcosm Study", May 26, 1995, BASF Report Number ER94066, BASF Corporation, Agricultural Products, P.O Box 13528, Research Triangle Park, NC 27709-3528.
3. Toreen A. Bixler, Huntingdon Analytical Services, "Freezer Storage Stability of BAS 300 I in Pond Sediment", December 7, 1994, BASF Report Number A9448, BASF Corporation, Agricultural Products Group, P.O. Box 13528, Research Triangle Park, NC 27709-3528

Figure 1. Flow Diagram for BASF Analytical Method No. D9201 as Applied to Pond Sediment

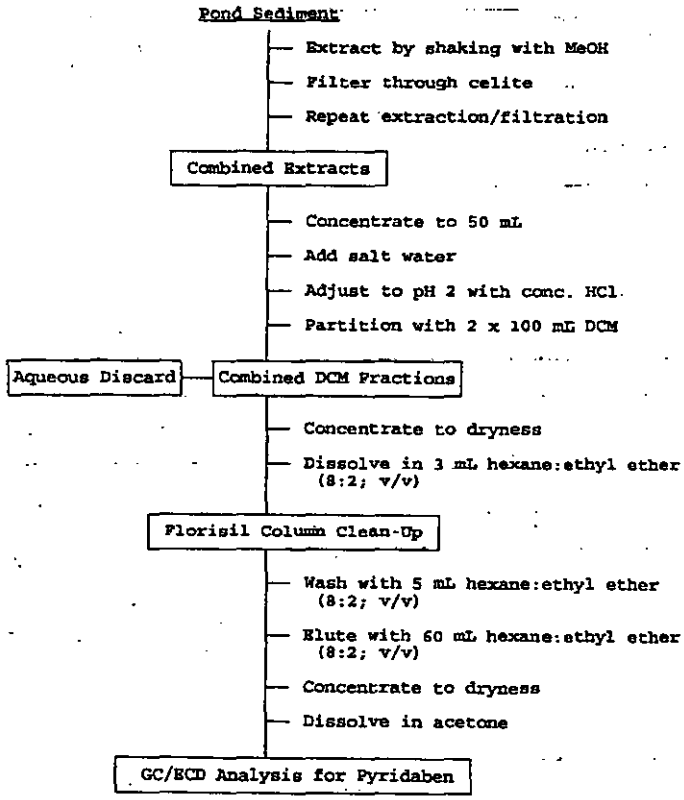


Figure 2. Typical Calculations for the Quantitation of Pyridaben in Pond Sediment

HAS Notebook/Queue Number: 4040603A; Pond Sediment fortified at 0.01 ppm with Pyridaben

PPM values were calculated using a multilevel calibration method that resides on a Perkin-Elmer LIMS/CLAS data acquisition system. The method constructed a weighted best fit line through identified calibration standards using the following equation:

$$y = cx + d$$

where: y = µg/mL Found
c = Slope (1.380E-5)
x = Peak Height Response (9728)
d = y - Intercept (-1.140E-2)

$$c = \frac{\Sigma \frac{1}{X_i} \Sigma Y_i - N \Sigma \left(\frac{Y_i}{X_i}\right)}{\Sigma \frac{1}{X_i} \Sigma X_i - N^2}$$

$$d = \frac{\Sigma X_i \Sigma \left(\frac{Y_i}{X_i}\right) - N \Sigma Y_i}{\Sigma \frac{1}{X_i} \Sigma X_i - N^2}$$

Four calibration standards were injected (in duplicate) throughout the analytical set from which the method was calibrated. The range of standards was 0.05 µg/mL to 0.50 µg/mL, 0.05 ng to 0.50 ng injected (See Figures 2-5). Using the analyte response (peak height) and the regression standard curve, the calibration method in the data acquisition system computed a concentration (Conc; µg/mL) for that analyte in the sample.

µg/mL	Peak Height
0.05	3862, 4474
0.10	8264, 9003
0.25	19916, 19538
0.50	36692, 35271

y = (1.380E-5)(9728) + (-1.140E-2)
y = 0.1228

The calculated Conc was then multiplied by the final volume (F.V.; identified as standard weight on chromatogram report), multiplied by a dilution factor (D.F.), if necessary, and divided by the sample weight (50 g) to obtain a ppm concentration based on the following equation:

$$ppm = \frac{Conc \times F.V. \times D.F.}{Sample\ Wgt}$$

Where:

Conc	=	µg/mL Found, (0.1228)
F.V.	=	5.0 mL
Sample Wgt	=	50.0 g

$$ppm = \frac{0.1228 \times 5 \times 1}{50}$$

$$ppm = 0.012$$

Recoveries were corrected for residues in controls as follows:

HAS Queue Number: 4040603A (Fortified Sample; see Figure 7)
4040601A (Control Sample; see Figure 6)

Corrected ppm = ppm Found in Fortified Sample - ppm Found in Control

= 0.012 - 0.002

= 0.010

% Recovery = $\frac{\text{Corrected ppm}}{\text{ppm Added}} \times 100$

= $\frac{0.010}{0.010} \times 100$

= 100