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THE CHEMISTRY AND ANALYSIS OF DIFLUBENZURON

AND ITS MAJOR METABOLITES

by

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## ABSTRACT

A new gas-liquid chromatographic (GLC) procedure has been developed for the analysis of diflubenzuron, 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea (Dimilin, TH 6040, PH 6040) and its major metabolites: 4-chloroaniline, 4-chlorophenylurea, and 2,6-difluorobenzoic acid in pond water. The residues from fortified pond water were extracted using ethyl acetate, and following derivatization, cleaned up on a micro Florisil column. Diflubenzuron and 4-chlorophenylurea were determined as their cleavage products, N-trifluoroacetyl-4-chloroaniline; 4-chloroaniline as its N-trifluoroacetyl derivative, and 2,6-difluorobenzoic acid as its O-pentafluorobenzyl derivative.

A 50% full scale deflection (fsd) was obtained with 0.03 ng of the N-trifluoroacetyl and pentafluorobenzyl derivatives. Recoveries from fortified unfiltered pond water for diflubenzuron, 4-chloroaniline, 4-chlorophenylurea and 2,6-difluorobenzoic acid at the 1.00, 0.10, and 0.02 mg/L levels were: 91.09, 75.02, 63.29%; 52.87, 22.60, 5.77%; 98.87, 84.10, 83.00%; 105.40, 105.44, and 101.94% respectively. Diflubenzuron and 4-chlorophenylurea were found to undergo cleavage during reaction with trifluoroacetic and heptafluorobutyric anhydrides. The products, N-monoperfluoroacyl-2,6-difluorobenzamide (from diflubenzuron) and N-monoperfluoroacyl-4-chloroaniline (from diflubenzuron and 4-chlorophenylurea) were identified as occurring during derivatization and not from thermal degradation of the 1- or 3-N-monoperfluoroacyl derivatives of diflubenzuron or 4-chlorophenylurea in the GLC injection port or column. Elucidation of a novel in vitro cleavage reaction and a proposed degradation scheme is presented based on EC-GLC, TC-GLC, TLC, GLC-MS and direct inlet probe-MS analysis.

The pentafluorobenzyl ester derivative of 2,6-difluorobenzoic acid was identified by GC-MS and proved to be a highly sensitive and stable derivative. 2,6-difluorobenzoic acid was extracted from acidified pond water (pH2) and reacted with pentafluorobenzyl bromide after conversion to the sodium salt of the acid.

Recoveries from filtered pond water for diflubenzuron were 94.10 and 89.30% at the 0.020 and 0.060 mg/L levels, respectively. 4-chloroaniline recoveries (filtered pond water) were 60.10 and 74.62% at the 0.02 and 0.10 mg/L fortification levels respectively. It is postulated that diflubenzuron and 4-chloroaniline are immobilized by adsorption, for example, to particulate organic matter in the pond water since recovery data from unfiltered pond water followed the linear form of the Freundlich equation.

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## I INTRODUCTION

Diffubenzuron, 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea (Dimilin, TH6040, PH6040) is a broad spectrum insecticide-larvicide (Thompson-Hayward Chemical Co; 1974) with a very low mammalian toxicity (acute oral LD<sub>50</sub> mice technical >4640 mg/kg). It is an insect growth regulator which interferes with the formation of insect cuticle (Post et al; 1974) when applied during the larval stages of many classes of insects. Extensive use is anticipated in the control of many domestic, agricultural and forest insects, such as mosquitoes, lepidopterous soybean insects, cutworms, grasshoppers, tussock moth, and spruce budworms.

Following application of diffubenzuron, residues may be present in aquatic systems via direct treatment of water or by runoff from treated areas into lakes, streams and ponds. Diffubenzuron is degraded in the environment to four major metabolites or degradation products. They are: 4-chloroaniline, 4-chlorophenylurea, 2,6-difluorobenzoic acid and 2,6-difluorobenzamide (Metcalf et al; 1975; Ruzo et al; 1974). A rapid, sensitive and reliable method is therefore required to enable analysis of diffubenzuron and its major metabolites in studies of their persistence and fate in aquatic systems. Since most laboratories rely on GLC for routine pesticide analysis, it was desirable to develop a sensitive GLC method for diffubenzuron and its major metabolites.

The purpose of this investigation was to study the chemistry and analysis of diffubenzuron and its metabolites from the environmental substrate pond water. The objectives of this study were as follows:

- (1) To develop a gas-liquid chromatographic method for the analysis of diffubenzuron and its metabolites 4-chloroaniline, 4-chlorophenylurea and 2,6-difluorobenzoic acid from fortified pond water.

(2) To elucidate the nature of the chemical reactions involved with diflubenzuron, 4-chloroaniline, and 4-chlorophenylurea in the presence of perfluoroanhydrides and 2,6-difluorobenzoic acid with pentafluorobenzyl bromide.

(3) To note the effect of pond water particulate matter on adsorption of diflubenzuron and 4-chloroaniline.

## II. LITERATURE REVIEW

Current Methodology for the Analysis of Diflubenzuron and its Major Metabolites.

Methodology for the analysis of diflubenzuron and its major metabolites may be described according to the following techniques and instrumentation used for separation and quantitation: Thin-layer chromatography (TLC), high-pressure (or performance) liquid chromatography (HPLC) and gas-liquid chromatography (GLC). Of some 122 papers published on diflubenzuron only 12 of these describe methodology for analysis; the remaining 110 papers being concerned with toxicological aspects. In addition, only 4 of the 12 methodology papers provide for analysis of one or more of the metabolites or degradation products of diflubenzuron.

## (a) Thin-Layer Chromatography:

Metcalf et al. (1975) studied the degradation and environmental fate of diflubenzuron in several systems: a model ecosystem, soil, microorganisms (Pseudomonas putida), sheep liver microsomes, and photodegradation using three radiolabelled versions of diflubenzuron (2,6-difluorobenzoyl ( $^{14}\text{C}=\text{O}$  label), p-chlorophenyl ( $^{14}\text{C}$ -ring-U) and a 2,6-difluorobenzoyl ( $^3\text{H}$ -ring) version. Extracts of the organisms or photodegradative reactions, analytical standards of diflubenzuron, and suspected metabolites or degradation products were subjected to TLC on fluorescent silica gel (E. Merck GF-254). The plates were developed in benzene-dioxane-acetic acid (90:30:1 by volume) and visualized by radioautography on no-screen x-ray film. No indication is given as to the method of quantitation but it was presumably accomplished by use of liquid scintillation spectrometry or a radiochromatogram scanner of

the TLC spots.

Maini and Desio (1976) analyzed diflubenzuron in the feces of the orchard insect Zeuzera pyrina using TLC. The extracts and analytical standard were spotted on silica gel G plates and developed in chloroform-ethyl-acetate-methanol (100:2:1). Detection with a  $\text{AgNO}_3$  reagent and UV light was used. The minimum amount of diflubenzuron detected was 1  $\mu\text{g}$ . Quantitation was based on spotting a series of standard concentrations and comparison with the unknown on the basis of Rf value and spot intensity.

(b) High Pressure Liquid Chromatography:

Corley et al. (1974) developed a method for the analysis of residues of diflubenzuron and its degradation products, 4-chloroaniline, and 2,6-difluorobenzamide from cows' milk using HPLC (coupled to an ultraviolet detector (UV) ) for separation and detection. Recoveries were essentially quantitative for diflubenzuron (0.1-1.0 mg/L) while recoveries were not reported for the two degradation products. The lowest detectable level (2x noise) for diflubenzuron was 10 ng at 254 nm and a flow rate of 0.2 ml/min. 2,6-Difluorobenzamide was well separated from diflubenzuron ( $R_T=44$  and 15.6 min respectively). However, 4-chloroaniline at a retention time ( $R_T$ ) of 10.2 min chromatographed with a tailing solvent peak.

Oehler and Holman (1975) reported an analytical method for the analysis of diflubenzuron from bovine manure at residue levels using  $\text{UV}_{254}$ -HPLC. A Florisil cleanup was also required prior to high pressure liquid chromatography. Recoveries ranged from 92 to 106% for manure fortified between 0.5 and 2.0 ppm. 100 Ng injected gave a 13% full scale deflection (fsd).

Several internal publications from Thompson-Hayward Chemical Co. (1975) followed much the same methodology as Oehler and Holman (1975). A number of different substrates were used for fortification with diflubenzuron and determination of recoveries employed UV-HPLC. At lower fortification levels (0.01 ppm) a Florisil cleanup was required prior to HPLC for water samples and Florisil or Florisil-alumina-silica gel for soil and forest samples (litter and foliage) and fish, eggs, milk or bovine tissues, respectively. The lower detection limit at 254 nm was 5 ng. Recoveries from water averaged 86% at fortification levels of 0.01 to 0.1 ppm, from soil and forest samples 90.7 and 80%, and from bovine tissues, fish, milk and eggs 90, 87.8, 85.8 and 79.7%, respectively. Duplicate analysis were not generally reproducible since variations of 10 and often 20% were reported. A final internal publication describes a UV-HPLC method for determination of active ingredient in technical Dimilin and 25% wettable powder; however, no detection limit, or recoveries were reported.

During an investigation to determine whether diflubenzuron accumulated in bovine tissues, manure or milk, Miller et al. (1976) followed the procedure of Corley et al. (1974) previously outlined. One modification was the use of a UV wavelength setting of 220 nm permitting a lower detectability limit of 5 ng cf 10 ng at 254 nm. When diflubenzuron was added to untreated feces at levels of 0.1, 0.5, and 1.0 ppm, recoveries averaged 94%, while recoveries from fat and muscle averaged 93 and 94%, respectively. The lower limit of detection was 0.1 ppm. Also fat and muscle tissues at 5 ppm were analyzed by the method of Oehler and Holman (1975) resulting in recoveries of 89 and 94%, respectively.

Finally, and most recently, Schaefer and Dupras, Jr. (1976)

reported a study on the persistence and stability of diflubenzuron and 4-chlorophenylurea in tap and field waters. UV<sub>254</sub>-HPLC was utilized for all quantitative analysis. No pre-column cleanup was used for any of the water samples (tap, pasture, pond, dairy drain, and sewage). Recoveries were generally high (90-100%) from tap, pasture and pond water and much lower for dairy drain and sewage waters (30-86%) at 0.01 to 0.10 mg/L levels. Their lower recoveries were attributed to the waters having a greater content of organic matter and hence loss through adsorption. Recoveries of 4-chlorophenylurea from tap water were 99, 100 and 95% at 0.1, 0.05 and 0.01 mg/L, respectively. No recoveries were reported for 4-chlorophenylurea from the other waters and the effects of particulate matter adsorption were not described. They found it was possible to estimate diflubenzuron concentrations to a detection limit of 0.001 ppm or 0.6 ng (minimum peak was twice background). Retention times were 5 and 7 min for diflubenzuron and 4-chlorophenylurea, respectively.

Although UV-HPLC has received considerable attention as an instrument for the separation and detection of diflubenzuron, 4-chloroaniline, and 4-chlorophenylurea, methodology based on GLC is favored for three main reasons:

(1) Much smaller sample sizes and extraction volumes can be used, e.g., 600 ml water samples/600 ml extraction solvent (Schaefer and Dupras, Jr., 1976) cf 50 ml water sample and 40 ml extraction solvent (Worobey & Webster, 1977) which reduces time (reduction of extraction volume to dryness) and cost.

(2) HPLC instrumentation is not yet commonly found in many pesticide monitoring and regulatory laboratories.

(3) HPLC detectors presently in use (UV) are inherently less sensitive than the electron capture (EC) detector commonly used with GLC.

(c) Gas-Liquid Chromatography:

Ruzo et al. (1974) reported the direct GLC of one of the major photodegradative/metabolic products of diflubenzuron, 2,6 difluorobenzamide. Using flame-ionization detection, and a 6 ft X 1/8 in i.d. glass column packed with 4% SE-30 (column temperature 170 degrees centigrade) a retention time of 2.3 minutes was reported. They did not describe detection limits since their study was basically qualitative.

The first GLC method for diflubenzuron residues was published by Lawrence and Sundaram (1976). The paper reports a GLC method for diflubenzuron after the compound is converted to a derivative amenable to GLC. Derivative formation for GLC has been found necessary since at normal GLC temperatures and on columns commonly used by pesticide residue analysts, chromatography was unsuccessful (Corley et al. 1974). The gas-liquid chromatographic analysis of diflubenzuron was carried out after the insecticide was converted to its N,N'-dimethyl analog (N-methyl-N-(4-chlorophenyl)-N'-methyl-N'-(2,6-difluorobenzoyl) urea). The methylation was accomplished in dimethyl sulphoxide (DMSO) with sodium hydride and methyl iodide. The derivative was thermally stable and chromatographed as a single peak on 3% OV-1 at 235°C. The sensitivity of the product to electrolytic-conductivity detection was approximately 50 ng for 50% full-scale deflection in the nitrogen mode and 70 ng for the same response in the chloride (reductive) mode. About 0.25 ng was required for 50% full-scale deflection by electron-capture detection. A method of extraction was described for diflubenzuron residues from spruce



foliage and as little as 0.2-0.5 ppm could be detected using the Coulson detector (nitrogen mode). Recoveries of diflubenzuron from fortified spruce foliage were generally greater than 80% above the 1.0 ppm fortification level. No information was given regarding electron-capture detection of foliage extracts.

Most recently Worobey and Webster (1977) described a GLC procedure developed for the analysis of diflubenzuron in pond water. Diflubenzuron residues were reacted with trifluoroacetic anhydride to produce a cleavage product, N-trifluoroacetyl-4-chloroaniline, and quantitated using electron capture detection. The derivative was found to be thermally stable and a 50% full scale deflection was obtained with 0.03 ng injected on a 5% OV-101 column at 122 degrees centigrade. Recoveries from fortified unfiltered pond water were 65.7, 72.8, 82.0 and 90.8% at the 0.020, 0.059, 0.197 and 0.788 ppm (mg/L) levels, respectively. Recoveries from fortified filtered pond water were 94.1 and 89.3% at the 0.020 and 0.060 mg/L levels, respectively. Trifluoroacetylation was chosen to provide a thermally stable derivative with increased volatility and enhanced electron-capturing properties.

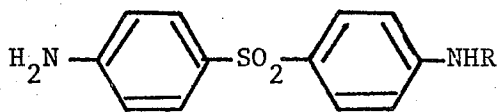
Another useful characteristic of the trifluoroacetyl derivative was that after derivatization the reaction mixture may be evaporated to dryness (to remove excess trifluoroacetic anhydride) without losses and this itself acted as a cleanup since the more volatile interferences were lost through evaporation while the derivative was retained.

#### In-Vitro Degradation of Pesticides and Drugs During Derivatization With Perfluoroanhydrides.

The use of derivatization techniques in the GLC of pesticides

or drugs is now widely accepted. The functional group transformations are usually straight-forward reactions carried out prior to injection into the chromatograph and often produce more thermally stable and detector-sensitive derivatives of the intact parent compounds. Perfluoroacylation of insecticidal carbamates (Magallona, 1975; Cochrane, 1975) and herbicidal ureas (Cochrane, 1975; Cochrane and Purkayastha, 1973; Saunders and Vanatta, 1974; Lawrence and Ryan, 1977; Ryan and Lawrence, 1977) as intact derivatives of the parent compounds have been previously reported and reviewed. With certain carbamates and ureas, however, perfluoroanhydrides may form unforeseen derivatives of the parent compound.

Vandenheuvel *et al.* (1973) investigated the reaction of heptafluorobutyric anhydride with a monosubstituted urea drug and a substituted carbamate drug. Need for an assay procedure to quantitate p-ureido-p'-amino-diphenyl sulfone (Ia) led them to consideration of



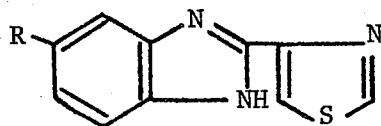
Ia, R = CONH<sub>2</sub>

Ib, R = H

hydrolysis of Ia to p,p'-diaminodiphenylsulfone (Ib) and conversion of this compound to a derivative suitable for electron-capture detection. They found that the reaction of Ib (hydrolysis product) with heptafluorobutyric anhydride resulted in the corresponding diheptafluorobutyramide. However, when Ia was reacted with heptafluorobutyric anhydride the product possessed the same retention time on the GLC as the authentic diheptafluorobutyramide of Ib. GLC-mass spectrometry demonstrated in both cases that the compound eluted from the GLC column was the same, namely, the diheptafluorobutyramide

of Ib. This remarkable conversion was not an "on column" alteration, since the product isolated from the reaction mixture possessed the same infrared and direct probe mass spectra as the diheptafluorobutyramide of Ib.

A somewhat related in-vitro cleavage reaction followed by concomitant conversion to a heptafluorobutyryl derivative was observed to occur during the reaction of the drug cambendazole (IIa)

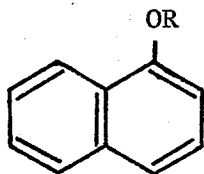


IIa, R =  $(\text{H}_3\text{C})_2\text{CHCONH}$

IIb, R =  $\text{NH}_2$

The reaction of IIa with heptafluorobutyric anhydride resulted in the formation of a mixture of mono and diheptafluorobutyramides of IIb. They then went on to prove that the formation of the mono and diheptafluorobutyramides of IIb did, in fact, occur as the result of an in-vitro cleavage reaction followed by subsequent conversion to these products.

Kalifa and Mumma (1972) and Mumma and Kalifa (1972) reported that N-hydroxymethylcarbaryl (IIIa) when reacted with trifluoroacetic



IIIa, R =  $\text{CONHCH}_2\text{OH}$

IIIb, R =  $\text{COCF}_3$

IIIc, R =  $\text{COF}_2\text{CF}_2\text{CF}_3$

or heptafluorobutyric anhydride yielded only the corresponding perfluoroacyl derivative of 1-naphthol (IIIb or c).

Adsorption of Diflubenzuron and 4-Chloroaniline.

Many excellent reviews of pesticide adsorption have been published (Bailey and White, 1964, 1970; Weber, 1971; S.C.I. Monograph, 1970). Adsorption of some phenylurea herbicides (Weber, 1971) on clays was postulated to occur by way of hydrogen bonds between the carbonyl oxygen atoms of the phenylureas and the water of hydration surrounding metal cations on the clay surface or through ion-dipole bonds between the carbonyl groups and the metal cations. Hance, (1969) suggested that the lack of any significant pH effect on the adsorption of some phenylureas was evidence that the compounds did not ionize and were probably adsorbed by physical Van der Waals forces.

Bailey et al. (1968) listed and discussed several possible mechanisms for phenylurea and aniline adsorption by clays and suggested that one or more may occur simultaneously, depending on the nature of the functional groups and the acidity of the system. Aniline was the most strongly adsorbed compound studied.

Stevenson (1972) presents a more recent review on adsorption of phenylurea herbicides by soils and especially organic matter. He lists and discusses Van der Waals, hydrogen-bonding, and ligand exchange as mechanisms for their adsorption.

(a) Adsorption of Chloroaniline Residues:

Bartha and Hsu (1976) reported that the bulk of chloroaniline residues derived from phenylamide herbicides are immobilized in spontaneous interactions with soil. Bailey et al. (1968) reported that of 50  $\mu$ moles aniline applied to a neutral and an acidic montmorillonite adsorbent, 21.4 and 39.0  $\mu$ moles were adsorbed based on their conditions of fortification and recovery. Bartha (1971) found that

4-chloroaniline fortified soil was bound to the soil more at 5 ppm than at 500 ppm (i.e. 77.4% cf 53.2%). This greater retention at lower levels is reflected in the extractable residues; 10.8% at 5 ppm and 40.3 % at 500 ppm. Hsu and Bartha (1974) reported that binding of 4-chloroaniline to sterile soil or purified humic acid was very rapid and at a 5 ppm level of fortification binding went to near completion (80%) within 27 hours. In addition, at 6 and 60 min, residues were bound at 25 and 55%, respectively.

(b) Adsorption of Diflubenzuron Residues:

Schaefer and Dupras, Jr. (1976) studied recoveries of diflubenzuron (at 0.10, 0.05 and 0.01 mg/L levels) from tap, pasture, pond, dairy drain and sewage lagoon waters. Reduced recoveries occurred in water having a greater content of organic matter, e.g., dairy drain and sewage water, presumably through adsorption. They also investigated recoveries of diflubenzuron (0.1 mg/L level) from tap water containing different amounts of straw (0-3 g/60.0 ml tap water) and held for 24 and 48 hrs at 21°C in the dark. The recoveries diminished as the amount of straw or the holding time increased. Extraction of the straw after 18 hr exposure showed that diflubenzuron was present and they concluded that the losses were partly, if not largely, due to adsorption. Carringer et al. (1975) studied the adsorption of diflubenzuron and other pesticides onto soil organic matter and concluded that adsorption appeared to be inversely related to the water solubility of the compound; they reported a water solubility of 0.3 ppm for diflubenzuron.

Finally, Worobey and Webster (1977) reported that diflubenzuron was very likely adsorbed by pond water organic matter. A plot of percent

recovery of diflubenuron from unfiltered pond water versus mg diflubenuron/L (fortification level) resulted in a curve, but when plotted as log concentration (mg/L) versus percent recovery, a straight line relationship was obtained. This was recognized as being similar to the linear form of the Freundlich equation (Khan, 1974):  $X = KC^n$ ; where  $X$  = amount of adsorbate taken up by a unit mass of the adsorbent,  $C$  = equilibrium concentration in solution,  $n$  = slope of the line,  $K$  = intercept of the isotherm. Analysis of their data by using the Freundlich equation gave a linear relationship. A value of  $n$  (0.710) was less than unity, indicating a convex, or L-type of isotherm (Weber, 1970). Apparently, this type of isotherm may arise due to a minimum competition of solvent for sites of the adsorbing surface or when the solid has a high affinity for the solute. Values of  $n$  that are less than unity have been reported for adsorption of various herbicides on humic acid, whereas montmorillonite gave a value of  $n$  greater than unity, indicating a concave or S-type isotherm (Weber, 1970).

## III. MATERIALS AND METHODS

The investigations reported in this manuscript included several individual studies. The analytical procedures employed or developed in the investigations are outlined below.

Reagents and Standards.

## 1. Sodium sulfate.

Reagent grade, anhydrous was extracted with hexane in a Soxhlet apparatus to remove organic contaminants, and washed with ethyl acetate prior to use.

## 2. Florisil.

60-100 mesh PR grade, Floridin Co; Pittsburg, PA., was activated at 620 degrees centigrade for 2.5 hr. and stored in a desiccator until cooled to room temperature.

## 3. Derivatization Reagents.

Trifluoroacetic anhydride, heptafluorobutyric anhydride and pentafluorobenzyl bromide were all obtained from Aldrich Chemical Company. Pentafluorobenzyl bromide reagent was prepared as a 1% (V/V) solution by dissolving 1ml reagent in 100ml acetone in a aluminum foil covered volumetric flask. (Caution: Reagent is a strong lachrymator.).

## 4. Sodium Carbonate (30% w/v).

Prepare a solution of 30g of anhydrous sodium carbonate (Macco, Reagent-A.C.S.) to 100ml with distilled water.

## 5. Sulphuric acid (10% v/v).

Prepare a solution of 10ml of concentrated sulphuric acid to 100 ml with distilled water.

## 6. Solvents.

Obtained from Caledon Chemical Co; Pesticide Grade-distilled in glass.

## 7. Pond Water.

Taken from artificial ponds (prepared by J. Madder of the Department of Entomology) at the Glenlea Research Station, Manitoba, and stored in a glass container until used.

## 8. Analytical standards.

Diflubenzuron (1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea) and its major degradation products, 4-chloroaniline, 4-chlorophenylurea, 2,6-difluorobenzamide, and 2,6-difluorobenzoic acid were supplied by Thompson Hayward Chemical Co., Kansas City, U.S.A. Structures and physical characteristics are listed in Table 1. All standards were recrystallized from methanol by dissolving in just enough methanol for solution plus three mls excess. The solution was heated to just boiling, filtered through glass-fiber filter paper, covered with a filter paper, and allowed to stand until crystallization occurred. The mother liquor was decanted off and the crystals washed with ca. 3 x 5 ml cold methanol and the crystals dried in a vacuum desiccator. 4-Chlorophenylurea was filtered through ca. 0.5 g Norit A, prior to heating of the methanol solution, to remove colored impurities. Melting points agreed with published data and their structures confirmed by direct inlet probe-mass spectrometry (70 eV ionization potential) using a Finnigan Model 1015 mass spectrometer. Standard solutions of the recrystallized standards were prepared in ethyl acetate and appropriate dilutions made as required.

### Apparatus

#### 1. Glassware.

Treated prior to use with Dri-film (SP5800, 15% in Toluene), a silanizing reagent, to prevent adsorption of residues and standards.



Table 1. Physical characteristics of diflubenzuron and its major metabolites.

| Compound  | Melting Point (°C) | Molecular Weight |
|---|--------------------|------------------|
| diflubenzuron<br>(1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea) | 239                | 310              |
| 4-chloroaniline   | 69                 | 127              |
| 4-chlorophenylurea  | 207                | 170              |
| 2,6-difluorobenzoic acid  | 159                | 158              |
| 2,6-difluorobenzamide   | 144                | 157              |

## 2. Gas Chromatograph.

Varian model 2440, equipped with tritium foil electron-capture detector. Operating conditions: temperatures (°C): injector 179, column 130 or 150, detector 182; nitrogen carrier-gas flow rate 40 ml/min.

## 3. GLC Column.

Pyrex (1.7 m x 2mm id) packed with 5% W/W OV-101 on 100-120 mesh Chromosorb W (AW) DMCS.

## 4. Gas Chromatograph - Mass spectrometer.

Dupont Dimaspec or Finnigan model 1015 equipped with column as described in 3. Operating conditions: temperature (C°): injector 180, column 150 or temperature program at 50-150 at 8°/min., separator 220, source 150 or 220; ionization potential 5, 7 or 70 ev; helium carrier gas flow rate 10 ml/min at separator exit.

## 5. Thin-Layer Chromatography (TLC).

TLC was carried out on 0.75 mm thick silica gel G plates (20 x 20 cm, pH 7) and developed in chloroform, ethyl acetate, acetic acid (85:10:15). Visualization was obtained using a very light spray of 0.01% fluoresceine in ethanol, followed by ultraviolet detection of spots. Elution of spots was carried out using ethyl acetate.

## 6. Thermal Conductivity-GLC.

Varian Aerograph (model A-90-P) with thermal conductivity detector, operating conditions: temperature (°C); injector 150, column 110, detector 153, collector 130, column; copper 21.6 cm x 4 mm (id) packed with 10% SE-30 on chromosorb W-AW (DMCS); nitrogen carrier gas flow rate 60 ml/min; collection tubes 10 cm x 2 mm (id) glass wrapped with pipe cleaners and cooled with acetone.

(a) Analysis of Diflubenzuron, 4-Chloroaniline, 4-Chlorophenylurea and 2,6-Difluorobenzoic Acid from Fortified Pond Water.

1. Fortification of Pond Water.

50 ml samples of pond water were transferred to a 125 ml glass stoppered separatory funnel and the water spiked with ca. 0.02, 0.10 and 1.00 mg/L of diflubenzuron, or 4-chloroaniline, or 4-chlorophenylurea, or 2,6-difluorobenzoic acid. 2.5 ml or less of the standard ethyl acetate solution was added in each case and the system allowed to equilibrate for 1 hr. "spiking" was done in duplicate for all standards.

2. Extraction of diflubenzuron, 4-chloroaniline and 4-chlorophenyl urea.

After the equilibration period described above, 2.5 ml 5% sodium chloride was added and the water extracted with 4 x 10 ml ethyl acetate. Each extraction was filtered through anhydrous sodium sulfate on Whatman glass fiber filter paper into a 100 ml round bottom flask.

The combined extract was taken to ca. 0.7 ml on a rotary evaporator at 30°C. The sample was transferred to a 5 ml graduated centrifuge tube with the aid of 3 x 0.5 ml ethyl acetate. The extract was then evaporated to 0.2 ml at 30 degrees centigrade under a low stream of nitrogen. Trifluoroacetic anhydride (0.2 ml) was added, the tube stoppered, and the contents mixed on a Vortex mixer, and the tube placed in a water bath at 50 degrees centigrade for 30 min. After the sample had reacted it was evaporated to dryness under nitrogen. At levels above 0.10 mg standard/L the sample could be diluted to an appropriate volume with ethyl acetate and 1.00 - 3.00

ul injected on-column into the GLC. Quantitation utilized the external standard method and peak height measurements.

### 3. Extraction of 2,6-difluorobenzoic acid.

Extraction of 2,6-difluorobenzoic acid was the same as extraction 2 described above except that after the equilibration period the pH of the water was adjusted to 2 with 10%  $\text{H}_2\text{SO}_4$  (V/V), checked with indicator paper and then 2.5 ml of 5% sodium chloride added. After extraction the extract was taken to dryness in a 30° water bath under a low stream of nitrogen. 200  $\mu\text{l}$  (about 2 drops) of 1% (V/V) pentafluorobenzyl bromide reagent, 10  $\mu\text{l}$  30%  $\text{Na}_2\text{CO}_3$  and 1 ml acetone were added to the dry residue. The tube was stoppered and reacted at 50° for 30 min in a water bath followed by evaporation to dryness under nitrogen, and made to an appropriate volume with hexane for EC-GLC injections. No Florisil cleanup was required at any of the fortification levels studied.

### 4. Cleanup.

For samples (except for 2,6-difluorobenzoic acid) containing less than 0.10 mg/L it was necessary to employ a Florisil column cleanup to remove interferences which produced severe tailing on the chromatogram of the derivatized standards. An 18 cm disposable Pasteur pipet was fitted with a small pledget of silanized glass wool and 6 cm (ca. 0.6 g) activated Florisil added and tamped to form the column. The derivatized sample in 0.5 ml ethyl acetate was transferred to the top of the column. The column was then eluted with 2.0 ml ethyl acetate and the eluent collected in a 5 ml glass stoppered centrifuge tube. The eluate was made up or reduced to (under nitrogen) a suitable volume and 1.00-3.00  $\mu\text{l}$  injected into the GLC.

(b) Analysis of Perfluoroacylation and Pentafluorobenzylation Reactions.

1. Preparation of derivatives

Diflubenzuron, 4-chlorophenylurea, 4-chloroaniline, or 2,6-difluorobenzamide (10-100  $\mu\text{g}$ /0.2 ml ethyl acetate in a 5 ml graduated glass stoppered centrifuge tube) was treated with trifluoroacetic or heptafluorobutyric anhydride (0.2 ml) at 50 degrees centigrade for 30 min in a water bath. The reaction mixture was then evaporated just to dryness under nitrogen and the residue taken up in an appropriate volume of ethyl acetate for analysis. For TC-GLC analysis 2 x 0.1 mg of diflubenzuron was reacted with trifluoroacetic anhydride as described above. Two major components were collected in acetone-cooled glass tubes inserted into the detector exit. The crystalline condensates of each were analyzed by DIP-MS. 2,6-difluorobenzoic acid was reacted as described above and its structure as a O-pentafluorobenzyl derivative confirmed by GLC-MS and direct inlet probe-mass spectrometry (DIP-MS).

(c) Effects of Pond Water Particulate Matter on Adsorption of Diflubenzuron and 4-Chloroaniline.

1. Filtered Pond Water Recoveries

Pond water (500 ml) was filtered through a 0.22  $\mu$  Microfil membrane filter under reduced pressure. The filtrate was then fortified at the 0.02 and 0.06 mg/L levels for diflubenzuron and 0.02 and 0.10 mg/L levels for 4-chloroaniline and analyzed in duplicate using the extraction method and cleanup described.

## IV. RESULTS AND DISCUSSION

(a) Perfluoroacylation of Diflubenzuron and Three Metabolites

In an attempt to extend the trifluoroacetic anhydride reaction previously reported for diflubenzuron (I) (Worobey and Webster, 1977) to three of its metabolites (see Figure 1), 4-chlorophenylurea (II), 4-chloroaniline (III) and 2,6-difluorobenzamide (IV), it was found that all three metabolites (II, III, IV) reacted producing single highly sensitive peaks, products IIC and IIIA having identical retention times by EC-GLC (Fig. 2). Reaction of diflubenzuron (I) with trifluoroacetic anhydride yielded two peaks, IB having the same retention time as reaction product IVA, and IC corresponding in retention time to reaction products IIC and IIIA (Fig 2). The use of several GLC columns of various polarities under a variety of column temperatures and flow rates failed to resolve peaks IC, IIC, and IIIA (see Appendix VIb).

As mentioned earlier the in vitro cleavage of certain ureas and carbamates in the presence of perfluoroanhydride derivatization reagents (trifluoroacetic and heptafluorobutyric anhydrides) has been demonstrated (Kalifa and Mumma, 1972; Vandenheuvel et al., 1973). Thus it was necessary to elucidate in this instance whether the reaction products of I and II were the result of on-column degradation or in vitro cleavage during the derivatization reaction.

The structural identity of all reaction products of I, II, III, and IV reacted with trifluoroacetic anhydride was determined using GLC-MS (see spectrums Appendix VIa). The total ion monitor chromatograms (Fig. 3) revealed four peaks from the reaction of I with trifluoroacetic anhydride having parent ions at  $m/e$  153(IA), 253 (IB),

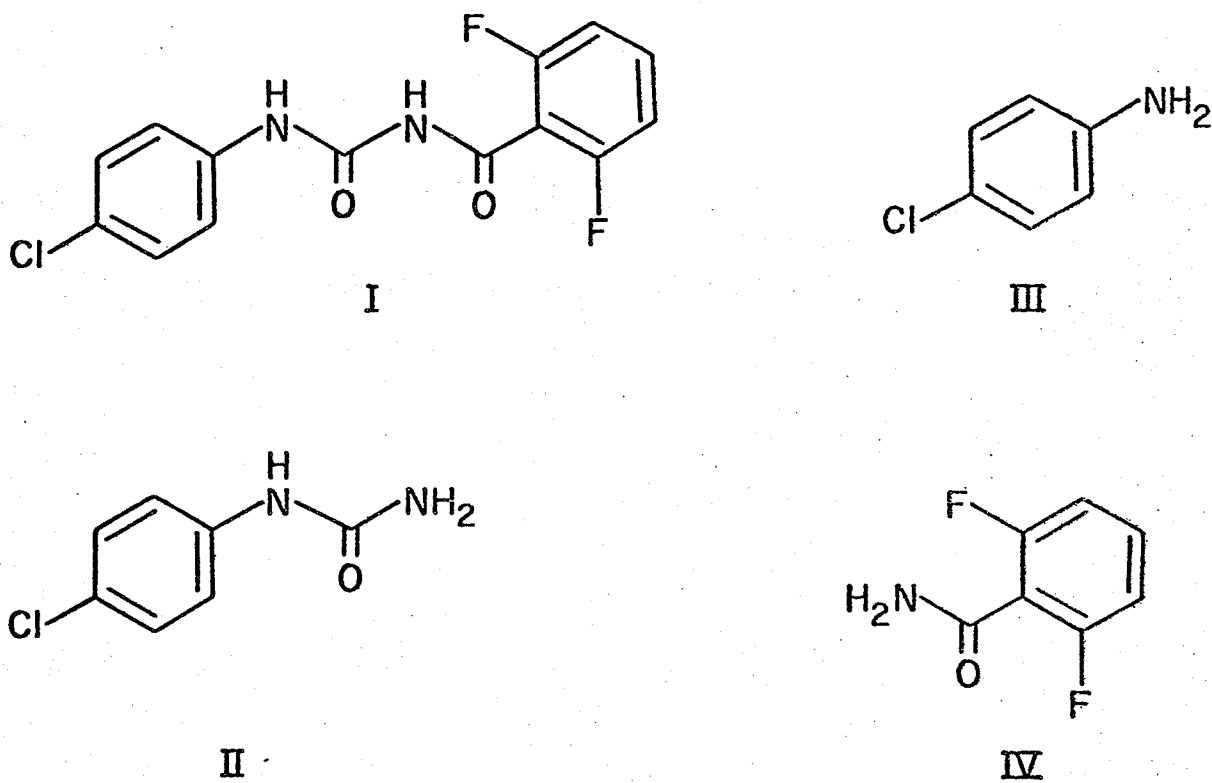


Figure 1. Structures of diflubenzuron (I), 4-chlorophenylurea (II), 4-chloroaniline (III), and 2,6-difluorobenzamide (IV).

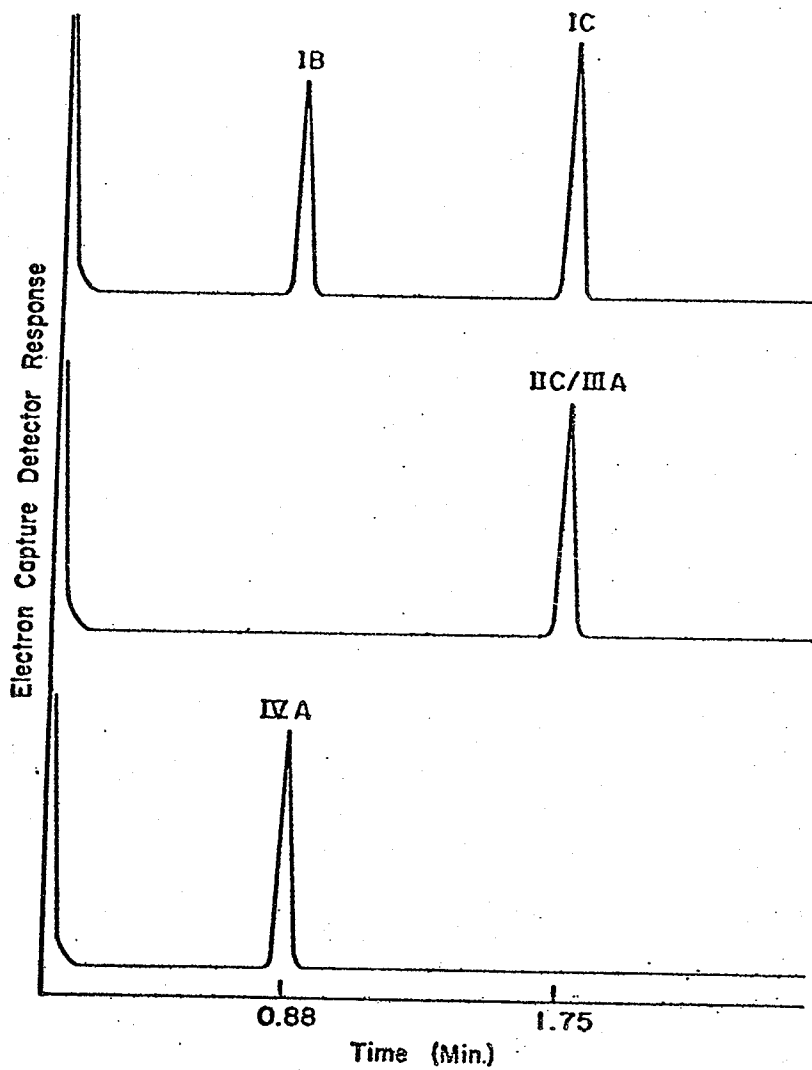


Figure 2. Gas chromatograms of products from the reaction of I, II, III, and IV with trifluoroacetic anhydride using EC-GLC.

Column conditions: 1.7 m x 2 mm id. glass column;  
5% OV-101 on Chromosorb W (AW) DMCS, 100-120 mesh;  
column temperature = 150°.



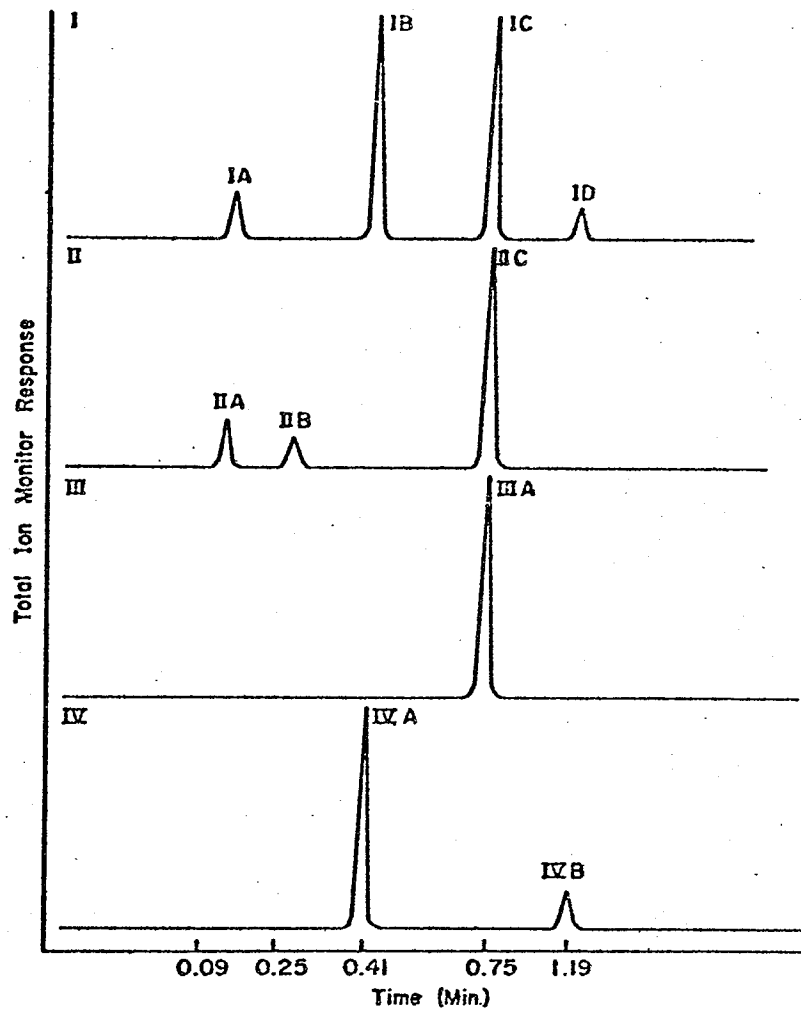


Figure 3. GLC-MS total ion monitor chromatograms of trifluoroacetic anhydride reaction products from I, II, III and IV.

GLC-MS conditions: 5% OV-101, 1.6 m x 2 mm id glass column;  
Ionization potential = 70ev, temp. prog. == 50-150° at  
8°/min.

223(IC), and 157(ID). II yielded three products with parent ions at m/e 153(IIA), 127(IIB), and 223(IIC); III, one product with m/e 223(IIIA); and IV, two products, with m/e 253(IVA) and 157(IVB). Table 2 lists daughter ions and their relative abundances for each of their products. The total ion monitor chromatograms indicated that IA and IIA (m/e 153), IIB (m/e 127) and ID and IVB (m/e 157) were minor components. Structural assignments for all parent ions are shown in Table 3.

Use of a thermal conductivity-gas liquid chromatograph (TC-GLC) facilitated the collection of each of the two major peaks IB and IC seen on EC-GLC since microgram or milligram amounts of compound may be injected and collected with an appropriate device at the detector exit. The two peaks collected corresponded to values of m/e 253 and 223 respectively. Thus, the two major peaks IB and IC from diflubenzuron as seen via EC-GLC were prepared and collected as primary standards yielding the same mass spectra as seen by GLC-MS of the reaction mixture.

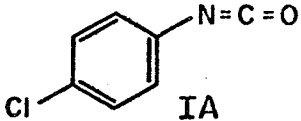
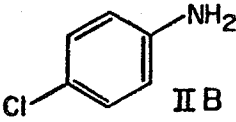
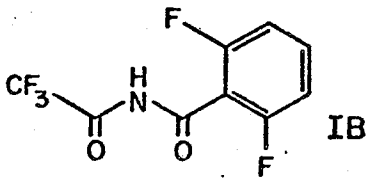
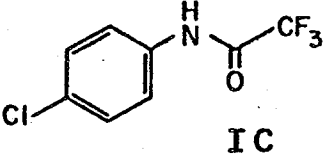
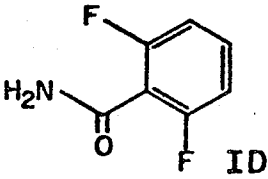
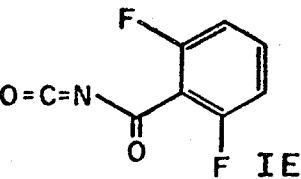
The fact that degradation of I and II had occurred during trifluoroacetylation was shown by TLC of the reaction products of compounds I-IV. Products from I and II yielded spots IC and IIC with the same R<sub>f</sub> (0.90) as product IIIA (Fig. 4). Elution and GLC-MS of these spots showed single chromatographic peaks for each with parent ions in each case at m/e 223. DIP-MS analysis at ambient temperature of the same material showed only m/e 223. It was evident that product IVA, the imide derivative, detected by GLC-MS with parent ion at m/e 253, had decomposed on the TLC plate since no IVA could be detected from the TLC separation of derivatized IV; the only compound detected had a parent ion at m/e 157 (IVB). In addition, the area from I (ID)

Table 2. The mass spectra of trifluoroacetyl derivatives of diflubenzuron and metabolites from Figure 3.

| TIM PEAK | COMPOUNDS <sup>a</sup> |       |        |       |        |        |        |        |       |       |
|----------|------------------------|-------|--------|-------|--------|--------|--------|--------|-------|-------|
|          | I                      |       |        |       | II     |        |        | III    | IV    |       |
|          | A                      | B     | C      | D     | A      | B      | C      | A      | A     | B     |
| m/e      |                        |       |        |       |        |        |        |        |       |       |
| 253      |                        | 23.1P |        |       |        |        |        |        |       | 34.2P |
| 225      |                        |       | 33.0   |       |        |        | 33.3   | 33.6   |       |       |
| 223      |                        |       | 100.0P |       |        |        | 100.0P | 100.0P |       |       |
| 157      |                        |       |        | 40.0P |        |        |        |        |       | 60.5P |
| 156      |                        |       | 13.1   |       |        |        | 9.6    | 10.7   |       |       |
| 155      | 33.3                   |       |        |       | 40.5   |        |        |        |       |       |
| 154      |                        |       | 39.8   |       |        |        | 30.6   | 32.8   |       |       |
| 153      | 100.0P                 |       |        |       | 100.0P |        |        |        |       |       |
| 141      |                        | 100.0 |        | 100.0 |        |        |        |        | 100.0 | 100.0 |
| 139      |                        | 3.1   |        |       |        |        |        |        | 4.14  |       |
| 129      |                        |       |        |       |        | 45.8   |        |        |       |       |
| 128      |                        |       | 33.5   |       |        |        | 24.7   | 27.1   |       |       |
| 127      | 17.8                   |       |        |       | 18.9   | 100.0P |        |        |       |       |
| 126      |                        |       | 47.7   |       |        |        | 34.6   | 37.3   |       |       |
| 125      | 53.3                   |       |        |       | 51.4   |        |        |        |       |       |
| 113      |                        | 22.3  | 5.7    | 50.0  |        |        | 3.4    | 3.5    | 32.9  | 31.6  |
| 111      |                        |       | 14.2   |       |        |        | 10.2   | 10.7   |       |       |

<sup>a</sup> Ions of less than 3% intensity or less than m/e 100 are not included.

Table 3. Products from the trifluoroacetylation reaction of compounds I, II, III, and IV detected by GLC-MS, and/or DIP/MS.

| Parent m/e | I   | II   | III   | IV   |
|------------|---|--|-------|------|
| 153, 155   | <br>IA   | IIA  | —     | —    |
| 127, 129   | —   | <br>IIB | —     | —    |
| 253        | <br>IB  | —  | —     | IV A |
| 223, 225   | <br>IC | IIC  | III A | —    |
| 157        | <br>ID | —  | —     | IV B |
| 183 *      | <br>IE | —  | —     | —    |

\* Product IE seen only on DIP - MS of reaction product of I

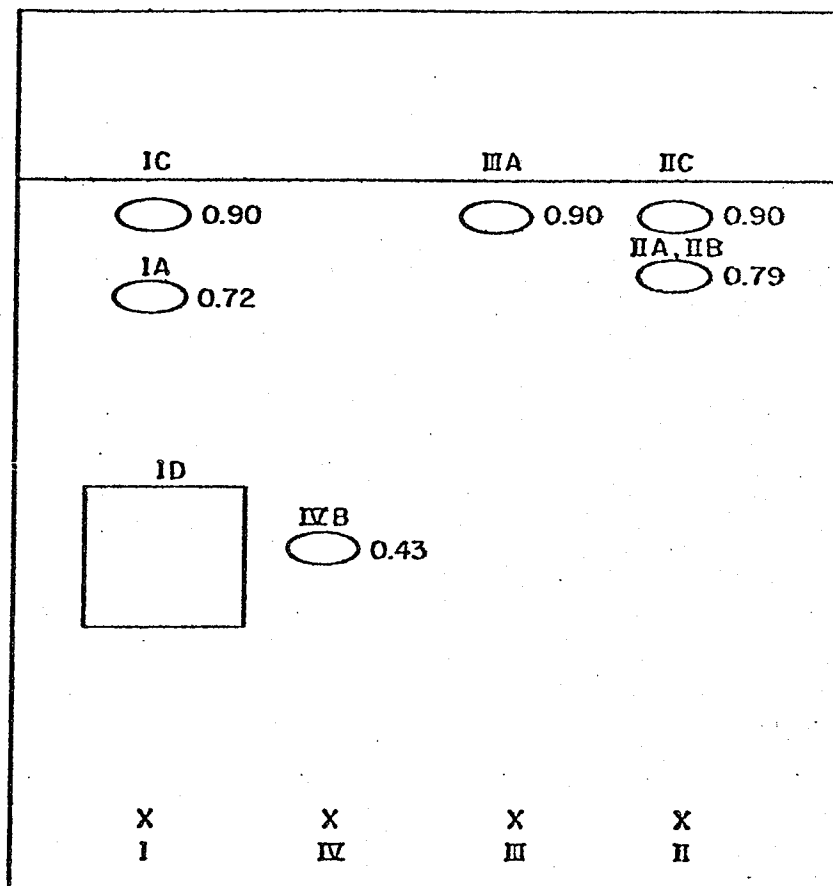


Figure 4. TLC of diflubenzuron (I) and its metabolites II, III, IV after reaction with trifluoroacetic anhydride.

with the same Rf (0.43) as spot IVB showed a parent ion at m/e 157. The fact that no IVA or IB could be isolated by TLC is to be expected since asymmetrical imides are very sensitive to hydrolysis in much the same way as asymmetrical anhydrides (Shemyakin et al., 1965), and the TLC plate was not activated. The GLC-MS data for all TLC spots are listed in Table 4.

Finally, as shown in Table 5, DIP-MS of the entire trifluoroacetic anhydride reaction product from I showed very minor peaks at m/e 406 (N-1- or N-3-trifluoroacetyl derivative of I) and m/e 502 (N,N-1, 3-ditrifluoroacetyl derivative of I). Intense parent ions at m/e 253, 223, and a strong peak at m/e 183 corresponding to 2,6-difluorobenzoylisocyanate were also evident. The product with parent m/e 183 was not detected by GLC-MS of the reaction products of I and therefore may arise in an ion-molecule reaction in the mass spectrometer, or as a product of the in vitro degradation reaction which does not elute from the GLC or elutes with a very long retention time. Ambient DIP-MS (7ev) analysis showed very strong m/e 253 and 223 confirming these ions as parent ions of the in vitro reaction products. Figure 5 shows the proposed degradation scheme for I and the subsequent formation of identified products. The reaction of isocyanates and carboxylic acids, e.g., trifluoroacetic acid, has been previously reported (Zabicky, 1970). The reaction pathway for II (4-chlorophenylurea) would be analogous to that for I (diflubenzuron, Fig. 5). The fact that the intensity of the m/e 406 peak was much less than m/e 253 or 223 was taken to indicate that it was not the parent ion for peaks at m/e 253 and 223. Previous mass spectra of substituted phenylureas have shown strong parent ions (Benson and Damico, 1968; Baldwin et al., 1968). In addition, the fact that the m/e 406 was not present

Table 4. The mass spectra of the TLC eluates from Figure 4.

| TIM PEAK | ELUATE <sup>a</sup> |        |       |        |        |        |        |        |
|----------|---------------------|--------|-------|--------|--------|--------|--------|--------|
|          | IVB                 | IA     | ID    | IC     | IIIA   | IIC    | IIA    | IIB    |
| m/e      |                     |        |       |        |        |        |        |        |
| 253      |                     |        |       |        |        |        |        |        |
| 225      |                     |        |       | 33.3   | 33.3   | 33.3   |        |        |
| 223      |                     |        |       | 100.0P | 100.0P | 100.0P |        |        |
| 157      | 60.5P               |        | 41.8P |        |        |        |        |        |
| 156      |                     |        |       | 4.2    | 4.2    | 3.7    |        |        |
| 155      |                     | 27.3   |       |        |        |        | 35.0   |        |
| 154      |                     |        |       | 12.5   | 12.5   | 11.0   |        |        |
| 153      |                     | 100.0P |       |        |        |        | 100.0P |        |
| 141      | 100.0               |        | 100.0 |        |        |        |        |        |
| 129      |                     |        |       |        |        |        |        | 42.9   |
| 128      |                     |        |       | 11.7   | 11.7   | 7.4    |        |        |
| 127      |                     | 9.1    |       |        |        |        | 15     | 100.0P |
| 126      |                     |        |       | 15.0   | 15.0   | 11.0   |        |        |
| 125      |                     | 36.4   |       |        |        |        | 35     |        |
| 113      | 31.6                |        | 20.9  |        |        |        |        |        |
| 111      |                     |        |       |        |        |        |        |        |

<sup>a</sup> Ions of less than 3% intensity or less than m/e 100 are not reported.  
 Ionization potential = 70 ev, GLC-MS, 5% ov - 101, column temp. = 150°C

Table 5. The mass spectra of the trifluoroacetyl derivatives of diflubenzuron (I) - Direct Inlet Probe - MS<sup>a</sup>.

| MS <sup>b</sup><br>IP <sup>c</sup> | DuPONT<br>70ev | FINNIGAN<br>70ev | FINNIGAN<br>7ev | FINNIGAN<br>5ev |
|------------------------------------|----------------|------------------|-----------------|-----------------|
| m/e                                |                |                  |                 |                 |
| 504                                | -              | 2                | 4               | 2               |
| 502                                | -              | 5                | 12              | 5               |
| 408                                | -              | 4.5              | -               | -               |
| 406                                | 0.5            | 13               | 3               | -               |
| 253                                | 200            | 2350             | 950             | 150             |
| 225                                | 440            | 1900             | 3100            | 400             |
| 223                                | 1290           | 5800             | 9000            | 1200            |
| 183                                | 240            | 3100             | 5100            | 500             |

<sup>a</sup> Ion intensity reported as millimeters peak height.

<sup>b</sup> MS = mass spectrometer, source temp = 150°C.

<sup>c</sup> IP = ionization potential



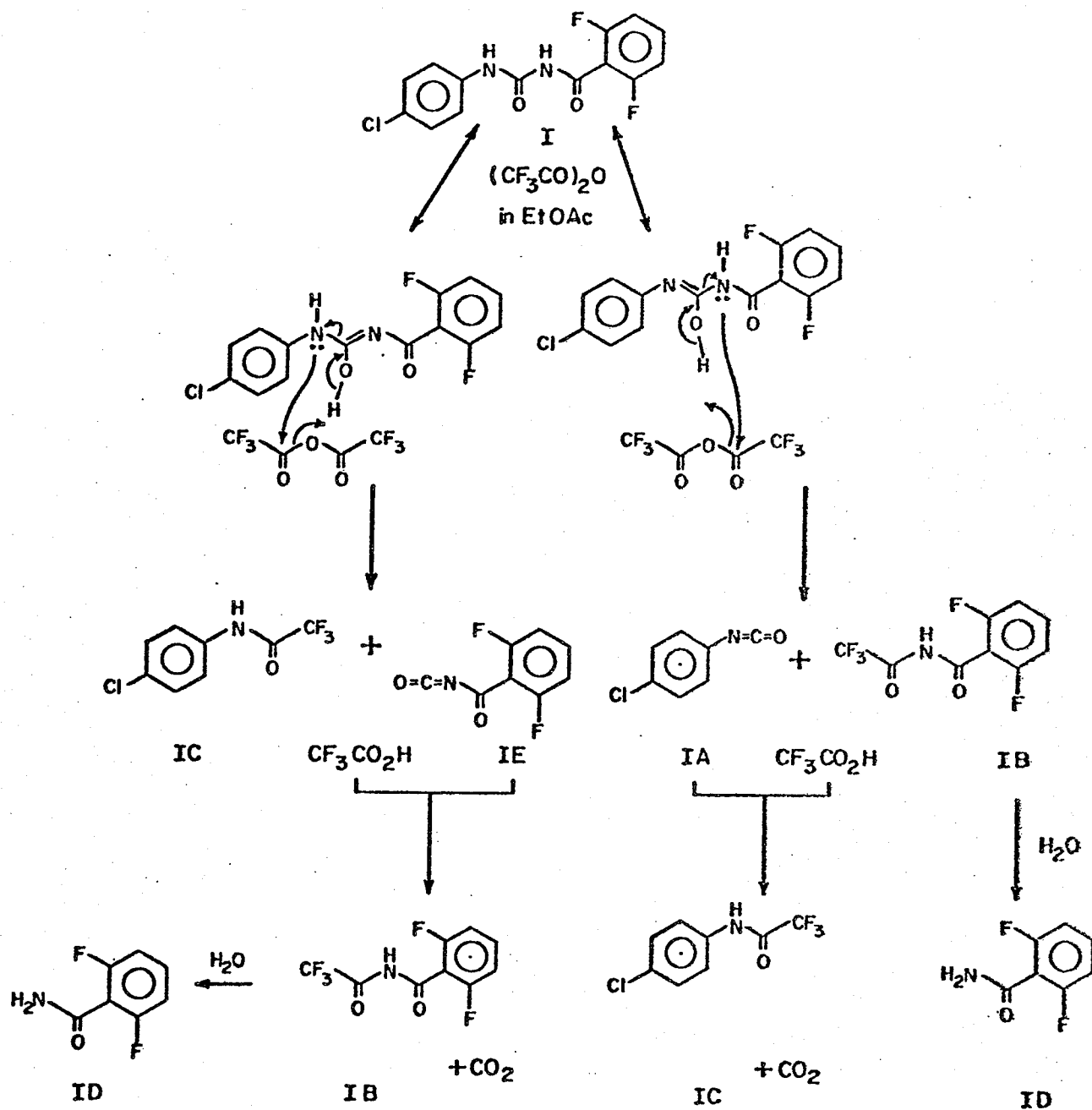


Figure 5. Suggested reaction pathways yielding products observed from trifluoroacetylation of diflubenzuron (I).

at 5 ev (Table 5) indicates that it was unlikely to be a parent ion. Fragmentation of either the mono- (m/e 406) or the di-trifluoroacetyl derivatives (m/e 502) could be expected to yield daughter ions with even mass numbers, i.e.; 252 and 222 respectively, unless a hydrogen atom rearrangement occurred. (There are none available to be rearranged in the di-derivative and it is unlikely than an N-H would be rearranged easily.) Thus it was concluded that m/e 253 and 223 were parent ions corresponding to products of an in vitro cleavage during the trifluoroacetylation step.

Heptafluorobutyrylation using heptafluorobutyric anhydride with I to IV gave analogous products to those obtained with trifluoroacetic anhydride with the two GLC peaks from I corresponding to m/e 353 and 323 (+100 = 2 x CF<sub>2</sub>).

Very little of the imide product from I or IV (m/e 353) was obtained via heptafluorobutyrylation compared with trifluoroacetylation. This is probably due to the even greater hydrolytic instability of a heptafluorobutyryl imide derivative.

With the trifluoroacetic anhydride reagent, the imide peak (IB or IVA) increased in intensity (EC-GLC) with time and temperature of the reaction. However, it decreased with prolonged evaporation to dryness, and on standing in ethyl acetate. No difference was observed whether 20 or 200  $\mu$ l of the derivatization reagents were used.

The importance of a careful investigation into the chemistry occurring during a derivatization step and subsequent GLC is evident. Indeed, a better understanding of these phenomena may lead to a more simplified and/or rational approach to analysis. For example, knowledge

that the urea moiety of I and II is cleaved during perfluoroacylation with concomitant conversion of the cleavage products to perfluoroamides eliminates the necessity for a separate hydrolysis step. By this method, diflubenzuron (I), and its metabolites, 4-chlorophenylurea (II) and 4-chloroaniline (III) can be simultaneously quantitated as the mono-N-perfluoroamide derivative of 4-chloroaniline (III). The method previously published for analysis of diflubenzuron in pond water as its N-trifluoroacetyl derivative, (Worobey and Webster, 1977), in fact proceeds via this pathway. Hydrolysis of herbicidal phenylureas to a substituted aniline product or carbamates to the corresponding phenol followed by derivatization is commonly used as an approach to residue analysis by GLC, (Cochrane and Purkayastha, 1973. Thus, when dealing with derivatization techniques, one must be alert to the possibility of the unexpected, during both the GLC and the derivatization steps.

(b) Pentafluorobenzoylation of 2,6-Difluorobenzoic acid

Pentafluorobenzoylation (using pentafluorobenzyl bromide) of 2,6-difluorobenzoic acid as its O- pentafluorobenzyl ether derivative proved to be a straightforward reaction. EC-GLC of the reaction product gave a single, symmetrical peak with a retention time of 25.63 minutes (column temperature: 130°). Confirmation of this peak by GLC-MS and DIP-MS yielded spectra showing a strong parent ion (M+) of m/e 338 and fragmentation ions of m/e 181 and m/e 141. (See spectrum Appendix VIA).

(c) Analysis of Diflubenzuron, 4-Chloroaniline, 4-Chlorophenylurea, and 2,6-Difluorobenzoic acid from Fortified Pond Water.

1. Analysis of diflubenzuron (I), 4-chlorophenylurea (II), 4-chloroaniline (III) as N-trifluoroacetyl derivatives of 4-chloroaniline.

Preliminary experiments to chromatograph I and II by direct GLC as the intact compound proved fruitless as no detector response could be obtained. These compounds were expected to be detectable using electron-capture (EC)-GLC (if no thermal decomposition occurred) via the presence of 3 and 1 halogen atoms respectively.

4-Chloroaniline (III) could be detected by EC-GLC; however, its peak was broad and sensitivity poor. Thermal instability of phenylureas has been attributed by earlier workers to the presence of the NH moiety (Saunders and Vanatta, 1974; Greenhalgh and Kovacicova, (1975)). The inability to chromatograph diflubenzuron (I) directly has been reported previously, (Corley et al., 1974; Worobey and Webster, 1977). Substitution of the -NH hydrogen to form a trisubstituted nitrogen or tetrasubstituted phenylurea appears to prevent thermal degradation (via GLC) of several phenylurea herbicides (Tanaka and Wein, 1973; Buchert and Lokke, 1975). The use of other derivatizing reagents including the NaH, CH<sub>3</sub>I, DMSO reaction for N-methylation of diflubenzuron as described by Lawrence and Sundaram, (1976), (see Appendix VIa of this thesis for a description of other derivatization reagents investigated) failed to yield any other useful derivatives of I, II or III.

Therefore, with an understanding of the reactions involving I, II and III when reacted with trifluoroacetic anhydride to produce N-trifluoroacetyl derivatives of 4-chloroaniline in each case, (as per section (a) of Results & Discussion), it was decided to use the

method of Worobey and Webster (1977) for analysis. Thus, I, II and III would be simultaneously analyzed for as the mono-N-trifluoroacetyl derivative of 4-chloroaniline (III). This in fact eliminates the need for a separate hydrolysis step since diflubenzuron and 4-chlorophenylurea are cleaved in vitro with concomitant conversion to N-trifluoroacetyl-4-chloroaniline derivatives. Hydrolysis of herbicidal phenylureas to a substituted aniline product or carbamates to the corresponding phenol followed by derivatization is commonly used as an approach to residue analysis by GLC (Cochrane and Purkayastha, 1973; Spengler and Hamroll, 1970; Magallona, 1975; Khan, 1975). As mentioned in part (a) of this section, the imide derivative of 2,6-difluorobenzamide was too unstable to permit quantitation and therefore, was not included in this study. Recovery data for I, II and III at approximately 1.0, 0.1, and 0.02 ppm fortification levels is given in Table 6. The linearity of the EC-GLC response to the mono-N-trifluoroacetyl derivatives of 4-chloroaniline derived from I, II and, 4-chloroaniline is shown in Figure 6. These compounds gave a linear response with increased concentration and quantitation was based on interpolation of sample peak heights within the linear range of each compound respectively. Figure 7 shows typical chromatograms for the derivatives of I, II, and III. Recoveries of diflubenzuron agreed with those reported earlier by Worobey and Webster (1977), (65.7, 72.8, 82.0 and 90.8% at 0.020, 0.059, 0.197 and 0.788 mg/L respectively) as well as recoveries from dairy drain water for diflubenzuron (analyzed intact by HPLC) published by Schaefer and Dupras, Jr. (1976) (76, 74 and 50% at 0.10, 0.05 and 0.01 ppm respectively). 4-Chlorophenylurea recoveries also agreed well with the Schaefer and Dupras, Jr. (1976) report.

Table 6. Recovery data for diflubenzuron, 4-chloroaniline, and 4-chlorophenylurea as their N-trifluoroacetyl-4-chloroaniline derivatives from fortified pond water.

| Compound           | Fortification Level |           | Recovery<br>μg/50 ml | Average | % Recovery |
|--------------------|---------------------|-----------|----------------------|---------|------------|
|                    | mg/L                | μg/50 ml. |                      |         |            |
| diflubenzuron      | 1.04                | 51.7      | 47.8                 | 47.1    | 91.0       |
|                    | 1.04                | 51.7      | 46.4                 |         |            |
|                    | 0.10                | 5.17      | 3.73                 | 3.88    | 75.0       |
|                    | 0.10                | 5.17      | 4.04                 |         |            |
|                    | 0.02                | 1.03      | 0.63                 | 0.65    | 63.2       |
|                    | 0.02                | 1.03      | 0.68                 |         |            |
|                    | 0.0                 | 0.0       | 0.0                  | 0.0     | 0.0        |
|                    | 4-chloroaniline     | 1.04      | 52.0                 | 27.7    | 27.4       |
| 1.04               |                     | 52.0      | 27.2                 |         |            |
| 0.10               |                     | 5.20      | 1.19                 | 1.17    | 22.6       |
| 0.10               |                     | 5.20      | 1.16                 |         |            |
| 0.02               |                     | 1.04      | 0.07                 | 0.06    | 5.77       |
| 0.02               |                     | 1.04      | 0.05                 |         |            |
| 0.0                |                     | 0.0       | 0.0                  | 0.0     | 0.0        |
| 4-chlorophenylurea |                     | 1.01      | 50.3                 | 51.8    | 49.7       |
|                    | 1.01                | 50.3      | 47.6                 |         |            |
|                    | 0.10                | 5.03      | 4.30                 | 4.23    | 84.1       |
|                    | 0.10                | 5.03      | 4.16                 |         |            |
|                    | 0.02                | 1.00      | 0.76                 | 0.83    | 83.0       |
|                    | 0.02                | 1.00      | 0.89                 |         |            |
|                    | 0.0                 | 0.0       | 0.0                  | 0.0     | 0.0        |

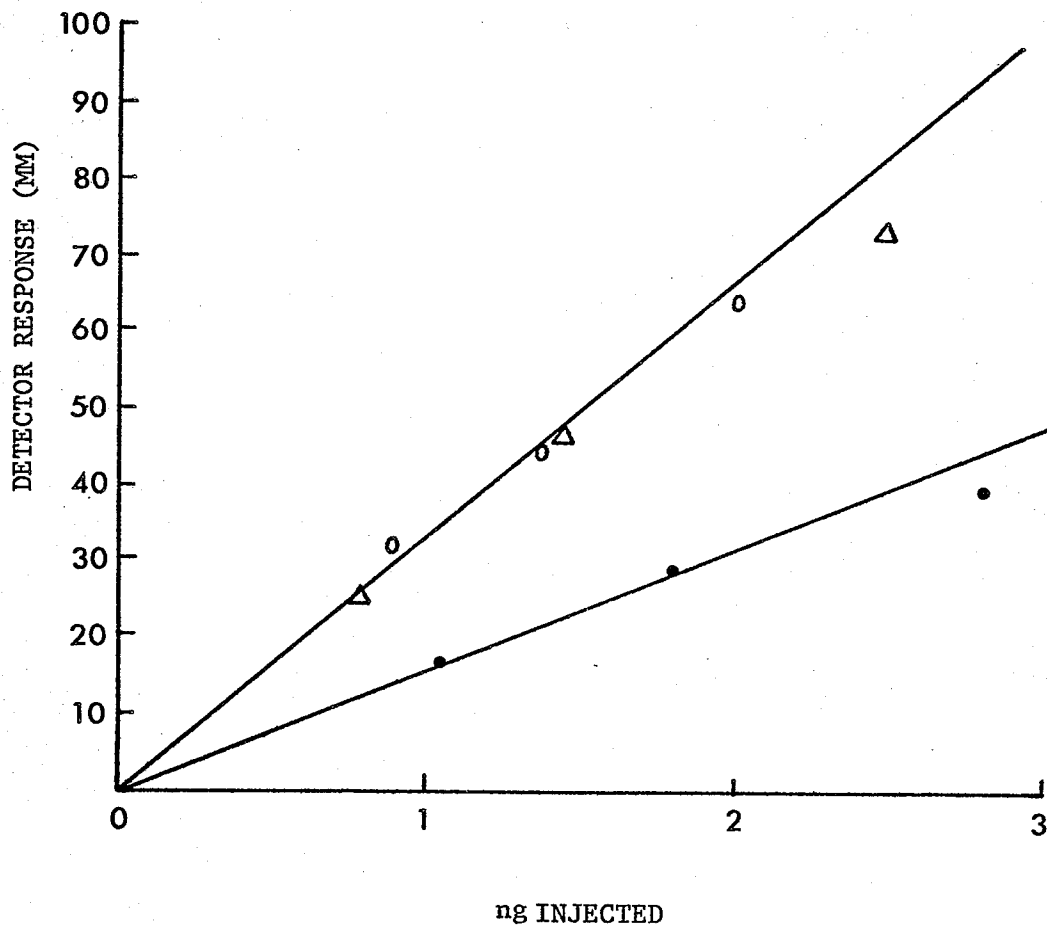


Figure 6. Ee-GLC linearity response to the N-trifluoroacetyl -4-chloroaniline derivatives from diflubenzuron (●), 4-chlorophenylurea (○), and 4-chloroaniline (△).

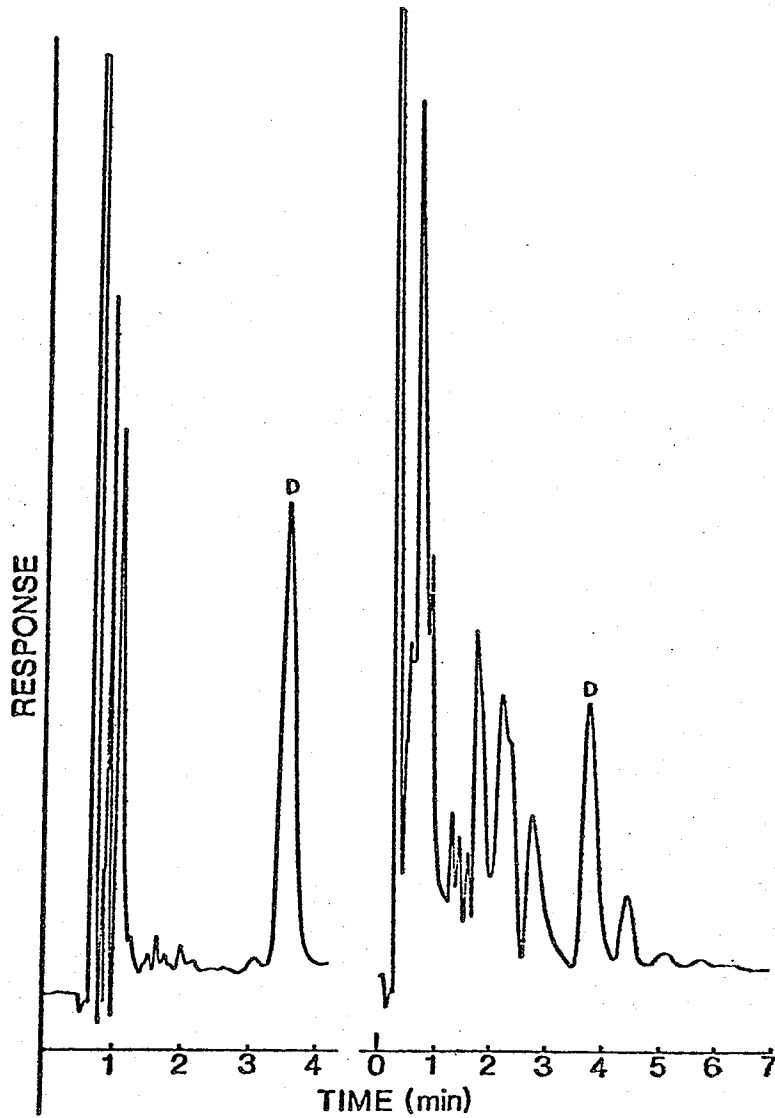


Figure 7. Chromatograms of N-trifluoroacetyl-4-chloroaniline standard (D) and extract from fortified unfiltered pond water at the 0.010 mg/L level.



They analyzed intact 4-chlorophenylurea in tap water at 0.10, 0.05 and 0.01 mg/L levels and found 99, 100 and 95% recoveries respectively. The somewhat lower recoveries (but higher cf diflubenzuron) may be attributed to partial adsorption to particulate matter in our pond water study. The lower recoveries for 4-chloroaniline (III) agree with results published by Hsu and Bartha (1974) who fortified sterile soil with 4-chloroaniline at 5 ppm and after 1 hour standing at room temperature recovered approximately 50% (cf 72.87) at 1.04 ppm level for this study).

The implications of these reduced recoveries with decreasing concentrations for fortification (for I and III) will be discussed in the following section (part c of Results and Discussion). Attempts were made to separate I, II and III from fortified pond water via acid (pH 1) or base (pH 10) extraction without success. The method developed, then, permits one to quantitate diflubenzuron, 4-chlorophenylurea and 4-chloroaniline simultaneously at subnanogram levels as the N-trifluoroacetyl derivative of 4-chloroaniline.

## 2. Analysis of 2,6-difluorobenzoic acid as its O-pentafluorobenzyl derivative.

Initial attempts to derivatize 2,6-difluorobenzoic acid 2,6-DFBA) with various derivatization reagents proved to be fruitless (see Appendix VIb). This problem was anticipated in that the ortho-fluoro groups may sterically hinder attack of the carboxylic OH group by derivatization reagents. Hyman (1969) has reported that only minimal conversion of 2,3,6-trichlorobenzoic acid to its methyl ester with tetramethyl ammonium hydroxide was almost certainly because of steric hindrance and it is also well

known that di-ortho substituted benzoic acids are resistant to all but the strongest methylating agents.

Norris and Bearse (1940) reported that the reaction of 2,6-dichlorobenzoic acid with thionyl chloride resulted in among the lowest velocity constants of a series of substituted benzoic acids, and that it was the presence of the ortho substituents that greatly reduced reactivity.

Metcalf et al., (1975) reported that the ortho groups of 2,6-DFBA may deactivate the benzoic acid to glycine conjugation. Chau and Terry (1975) studied the derivatization of 2,3,6-trichlorobenzoic acid with  $\text{BCl}_3/2$ -chloroethanol in various solvents, but conversion to the 2-chloroethyl derivative was very small or not observable. They found however, that reaction with dicyclohexyl carbodiimide/2-chloroethanol yielded higher recoveries of the 2-chloroethyl derivative. This reaction was investigated with 2,6-DFBA and although a peak was obtained that was not present in the reagent blank its identity as a 2-chloroethyl derivative could not be confirmed by GLC-MS.

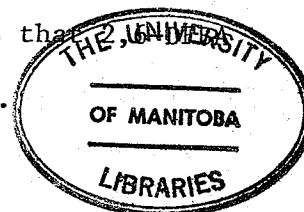
The use of diazomethane had proven most successful for preparation of a methyl ester derivative of di-orthohalo benzoic acids (Mattson and Kirtsen, 1968 (2,3,6-trichlorobenzoic acid, 2,3,6-TCBA), Yip, 1971, (2,3,6-TCB), Kirkland, 1961, (2,3,6-TCBA), and Muelmans and Upton, 1966 (2,6-dichlorobenzoic acid) ). The reaction of diazomethane with 2,6-DFBA was tried and resulted in formation of the methyl ester (identified by GLC-MS); however, sensitivity was too low for use of this derivative at subnanogram levels.

Attention was then turned to the derivatization reagent pentafluorobenzylbromide (1-bromomethyl-2,3,4,5,6 pentafluorobenzene).

Its reaction had been reported with 2,3,6-TCBA resulting in a symmetrical peak (via EC-GLC) and good sensitivity (0.1ng gave ca a 40% full-scale deflection, column temperature 200 degrees centigrade) (Chau and Terry, 1976). Several other investigators reported the successful derivatization of herbicidal acids (Agemian and Chau, 1976; Johnson, 1973), the phenolic hydrolysis products of N-methylcarbamates (Coburn and Chau, 1974, 1975), organic acids (Kawahara, 1968, 1971) and phenols and mercaptans (Kawahara, 1968, 1971).

The reaction conditions described by Chau and Terry (1976) were then applied to a sample of 2,6-DFBA resulting in an EC-GLC (column temperature 130 degrees centigrade) peak with good symmetry and excellent sensitivity; 0.5 ng gave a 23% full scale deflection at attenuation 64. To shorten the methodology for the reaction somewhat, the following modifications were made giving the same peak (retention time = 25.63 min) and sensitivity (25% fsd/0.5 ng injected, attenuation 64). 10  $\mu$ l of 30%  $\text{Na}_2\text{CO}_3$  was used in place of 30  $\mu$ l  $\text{K}_2\text{CO}_3$  (to decrease co-extracted interferences); the sample was reacted at 50 degrees centigrade for 30 minutes (to shorten the reaction time from 5 hours at room temperature and permit the same reaction time as for trifluoroacetylation) and evaporation of the sample to dryness under nitrogen after reaction (cf a benzene extraction of an aqueous layer and drying of benzene over anhydrous sodium sulfate).

The modified method of derivatization is as described under Materials and Methods of this manuscript. The method of analysis was essentially the same as that described for diflubenzuron and its metabolites except that the pond water is acidified to pH 2 or less with 10%  $\text{H}_2\text{SO}_4$  prior to extraction with ethylacetate. This assures that is in the free acid form for extraction into ethyl acetate.



Recovery data at three levels from fortified pond water are shown in Table 7. Recoveries were excellent at 105.4, 105.4 and 101.9% at the 1.03, 0.10 and 0.02 mg/L levels of fortification respectively. The linear range of the electron capture detector to the 0-pentafluorobenzyl derivative of 2,6-DFBA standard was 0-1 ng (Figure 8).

(d) Effects of Pond Water Particulate Matter on Adsorption of Diflubenzuron and 4-Chloroaniline.

An analysis of the recoveries of diflubenzuron and 4-chloroaniline yielded some interesting results concerning their possible adsorption by pond water particulate matter. The pond water may be expected to contain particles of soil, sand, silt, clay, organic matter (in various states of humification) and raw vegetative matter, all of which may adsorb organic compounds (see Literature Review re adsorption). In addition the presence of very fine particles in suspension would be expected to provide a large surface area for adsorption to take place.

A plot of log mg of compound/L versus percent recovery resulted in a straight line relationship for 4-chloroaniline and diflubenzuron (Figure 9). This was recognized as being similar to the linear form of the Freundlich equation (Khan, 1974),  $X = KC^n$  where: X = amount of adsorbate taken up by a unit mass of adsorbent; C = equilibrium concentration in solution; N = slope of the line; and K = intercept of the isotherm.

An analysis of the data using the Freundlich equation gave a linear relationship conforming to the equation (Table 8, Figure 10). The values of n were 0.860 and 0.625 for 4-chloroaniline and diflubenzuron respectively. Values of n were less than unity indicating a convex, or L-type isotherm (Weber, 1970). This type of isotherm may arise due to a minimum competition of solvent for sites on the adsorbing surface or when the solid has a high affinity for the solute. Values

Table 7. Recovery data for 2,6-difluorobenzoic acid and its 0-pentafluorobenzyl derivative from fortified pond water.

|                             | Fortification Level |          | Recovery<br>µg/50 ml | Average<br>µg/50 ml | % Recovery |
|-----------------------------|---------------------|----------|----------------------|---------------------|------------|
|                             | mg/L                | µg/50 ml |                      |                     |            |
| 2,6-difluorobenzoic<br>acid | 1.03                | 51.5     | 55.5                 | 54.2                | 105        |
|                             | 1.03                | 51.5     | 53.0                 |                     |            |
|                             | 0.10                | 5.15     | 5.25                 | 5.43                | 105        |
|                             | 0.10                | 5.15     | 5.60                 |                     |            |
|                             | 0.02                | 1.03     | 1.11                 | 1.05                | 101        |
|                             | 0.02                | 1.03     | 0.99                 |                     |            |
|                             | 0.0                 | 0.0      | 0.0                  | 0.0                 | 0.0        |
|                             | 0.0                 | 0.0      | 0.0                  | 0.0                 | 0.0        |

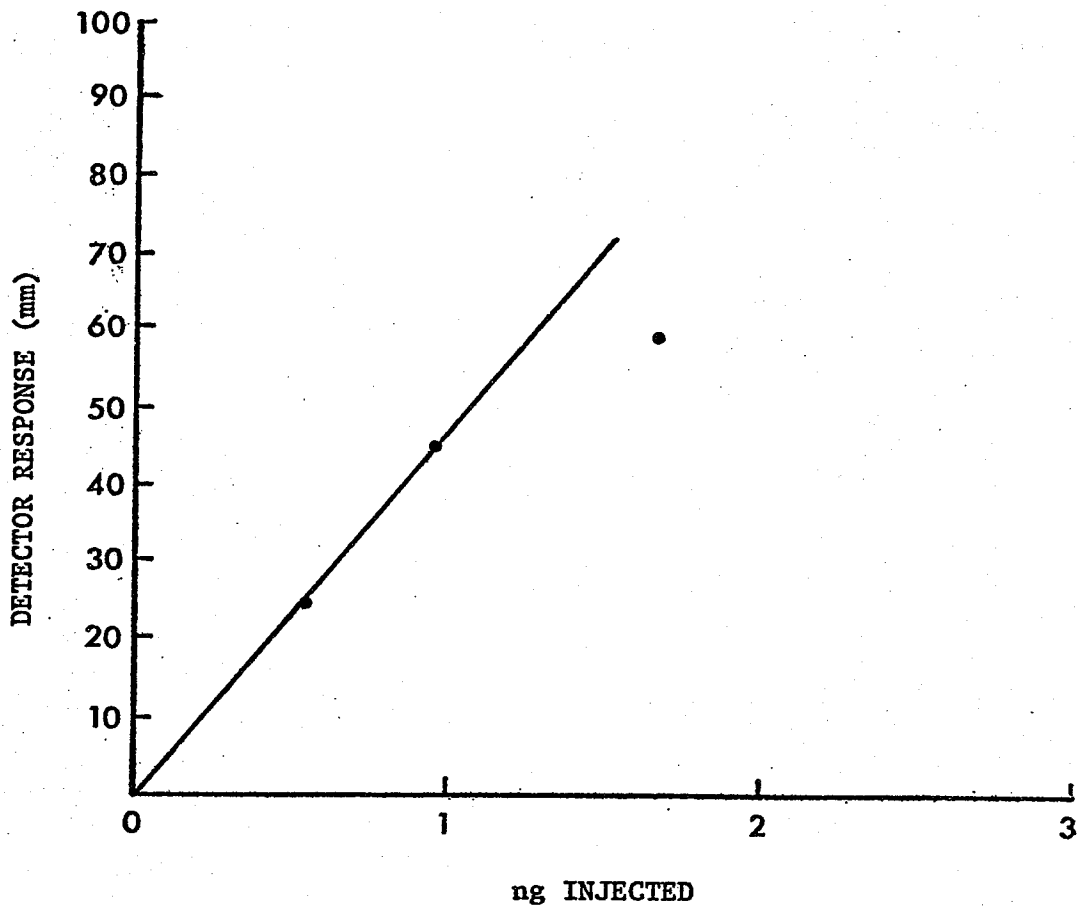


Figure 8. EC-GLC linearity response to the O-pentafluorobenzyl derivative of 2,6-difluorobenzoic acid.

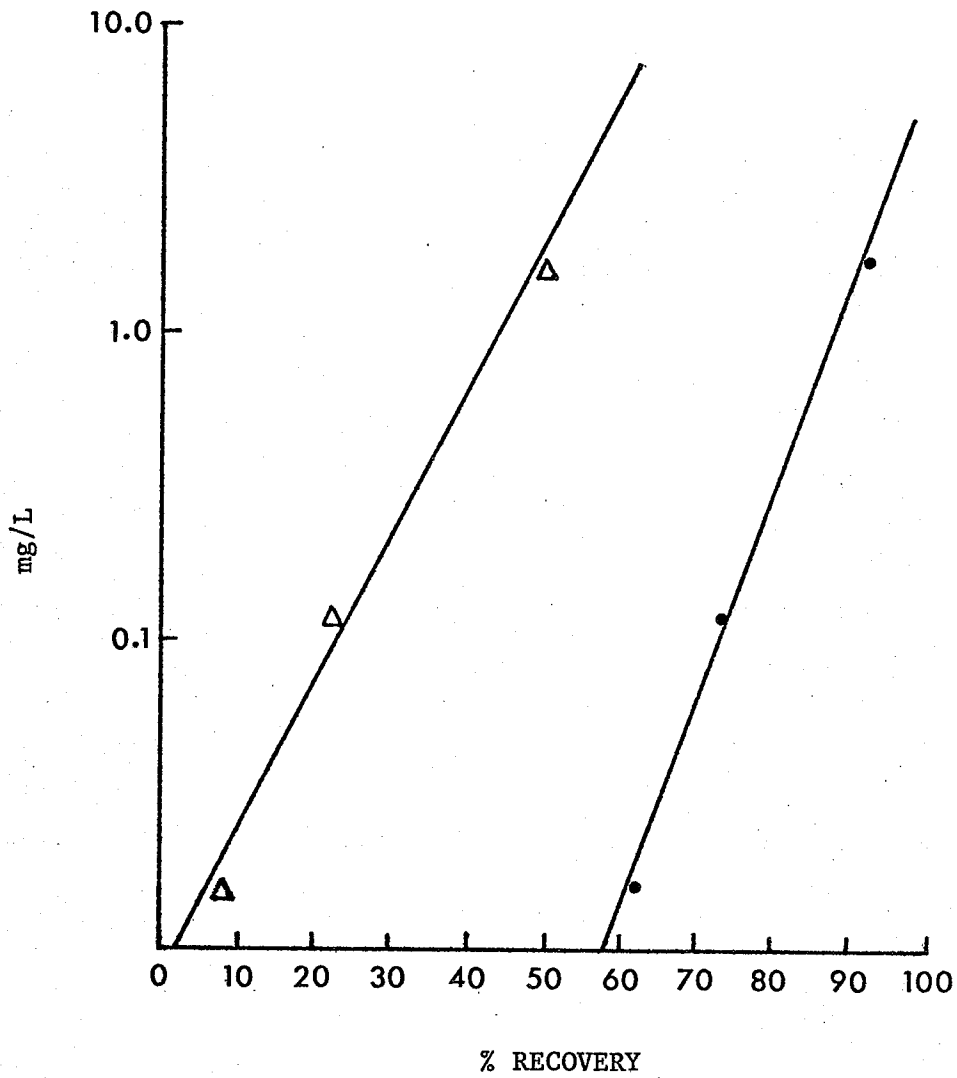


Figure 9. Log mg/L versus % recovery for 4-chloroaniline ( $\Delta$ ), diflubenzuron ( $\bullet$ ).

Table 8. Data re. Freundlich equation for adsorption of diflubenzuron and 4-chloroaniline by 50 ml. unfiltered pond water.

| Compound        | A = $\mu\text{g}$ Added | R = $\mu\text{g}$ Recovered | X = (A - R) |
|-----------------|-------------------------|-----------------------------|-------------|
| diflubenzuron   | 51.7                    | 47.1                        | 4.60        |
|                 | 5.17                    | 3.88                        | 1.29        |
|                 | 1.03                    | 0.65                        | 0.38        |
|                 | 0.0                     | 0.0                         | 0.0         |
| 4-chloroaniline | 52.0                    | 27.4                        | 24.5        |
|                 | 5.20                    | 1.17                        | 4.03        |
|                 | 1.04                    | 0.06                        | 0.98        |
|                 | 0.0                     | 0.0                         | 0.0         |



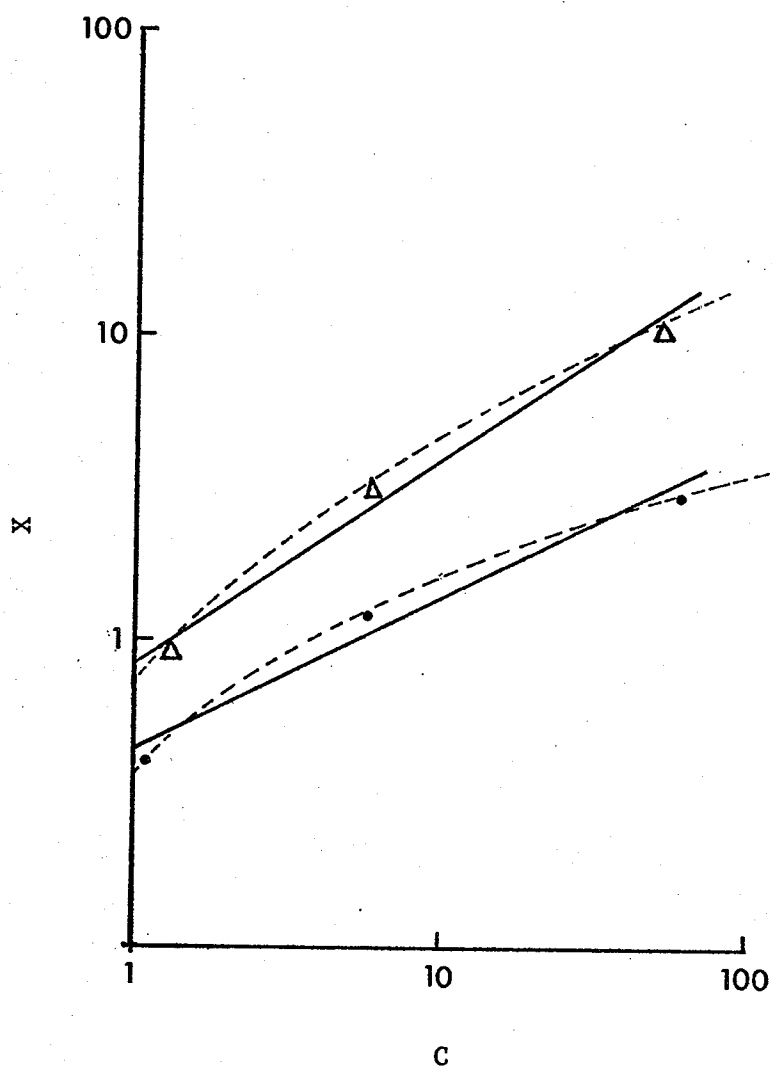


Figure 10. Isotherms for diflubenzuron (•) and 4-chloroaniline (Δ) generated from data analyzed using the Freundlich equation.

of  $n$  that are less than unity have been reported for adsorption of various herbicides on humic acid, whereas, montmorillonite gave a value of  $n$  greater than unity, indicating a concave or S-type isotherm (Weber, 1970). The values of  $n$  in this case are less than 1 indicating that adsorption involves organic matter more than clay particles. The value of  $n$  for diflubenzuron in this study (0.625) agreed well that reported recently by Worobey and Webster (1977) of 0.710. Using the Freundlich "K" as a criterion of extent of adsorption (Bailey et al., 1968) one can easily see that 4-chloroaniline is more readily adsorbed than diflubenzuron; K equals 0.820 and 0.385 respectively.

Finally, the recoveries of diflubenzuron and 4-chloroaniline from filtered pond water are listed in Table 9. The improved recoveries for both compounds confirm the existence of adsorptive immobilization of diflubenzuron and 4-chloroaniline residues. These results for diflubenzuron agree with those of Carringer et al. (1975) who found that diflubenzuron was strongly adsorbed to organic matter. They also reported that compounds of low water solubility (diflubenzuron = 0.3 mg/L) are preferentially adsorbed to hydrophobic areas (e.g., suspended organic matter) and removed from solution. Desorption studies added further evidence that hydrophobic bonding may be involved in adsorption of diflubenzuron since it was less easily desorbed from the organic matter of compounds of greater water solubility.

Table 9. Recoveries of diflubenzuron and 4-chloroaniline from filtered pond water.

|                 | Fortification Level |          | Recovery<br>µg/50 ml | Average<br>µg/50 ml | % Recovery |
|-----------------|---------------------|----------|----------------------|---------------------|------------|
|                 | mg/L                | µg/50 ml |                      |                     |            |
| 4-chloroaniline | 0.0                 | 0.0      | 0.0                  | 0.0                 | 0.0        |
|                 | 0.02                | 1.04     | 0.64                 | 0.63                | 60.1       |
|                 | 0.02                | 1.04     | 0.61                 |                     |            |
|                 | 0.10                | 5.20     | 3.85                 | 3.88                | 74.6       |
|                 | 0.10                | 5.20     | 3.91                 |                     |            |
| diflubenzuron   | 0.0                 | 0.0      | 0.0                  | 0.0                 | 0.0        |
|                 | 0.02                | 1.00     | 0.88                 | 0.94                | 94.1       |
|                 | 0.02                | 1.00     | 1.00                 |                     |            |
|                 | 0.06                | 3.00     | 2.80                 | 2.68                | 89.3       |
|                 | 0.06                | 3.00     | 2.57                 |                     |            |

## V. CONCLUSION AND SUMMARY

The acceptance of a new pesticide for use in our environment is only complete once a method for the parent compound and its major degradation and metabolic products is available at residue levels. Since most pesticide monitoring and regulatory laboratories depend on the use of the gas-liquid chromatograph to separate and detect these residues, a rapid, reliable, and sensitive method was required for the analysis at residue levels of diflubenzuron and its major degradation and metabolic products, 4-chloroaniline, 4-chlorophenylurea and 2,6-difluorobenzoic acid. In an attempt to develop such a method, several investigations were conducted.

An investigation was conducted to gain a thorough understanding of the nature of the trifluoroacetic anhydride reaction with diflubenzuron and its metabolites. Diflubenzuron (1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea) and one of its metabolites, 4-chlorophenylurea were found to undergo cleavage during reaction with trifluoroacetic and heptafluorobutyric anhydrides. The products, N-monoperfluoroacyl-2,6-difluorobenzamide (from diflubenzuron) and N-monoperfluoroacyl-4-chloroaniline (from diflubenzuron and 4-chlorophenylurea) were identified as occurring during derivatization and not from thermal degradation of the 1- or 3-N-monoperfluoroacyl derivatives of the intact diflubenzuron in the GLC injection port or on the GLC column. Elucidation of an in vitro cleavage reaction and a proposed degradation scheme was presented based on EC-GLC, TC-GLC, TLC, GLC-MS and direct inlet probe-MS analysis.

An investigation of the suitability of a published GLC method (for diflubenzuron) for simultaneous analysis of 4-chloroaniline,

4-chlorophenylurea and diflubenzuron from pond water was conducted. Diflubenzuron, 4-chloroaniline and 4-chlorophenylurea were quantitated at residue levels from fortified pond water as N-trifluoroacetyl-4-chloroaniline derivatives. EC-GLC provided a most sensitive method for detection (0.03 ng gave a 50% full scale deflection). Compared with methods previously published for analysis using UV-HPLC, the EC-GLC method was found to be more rapid and less costly since the method uses small scale glassware, requiring less than 50 ml solvent per 50 ml sample. The method allows quantitation of total diflubenzuron + 4-chloroaniline + 4-chlorophenylurea as N-trifluoroacetyl-4-chloroaniline. Reproducibility of the method was good with recoveries decreasing at lower levels, these lower recoveries being attributed to the adsorption and not poor efficiency of the method.

A method was also developed for 2,6-difluorobenzoic acid residues by modifying the one used to analyze for diflubenzuron, 4-chloroaniline and 4-chlorophenylurea. The fortified pond water was acidified to pH 2 or less to convert 2,6-difluorobenzoic acid to its free acid prior to extraction into ethyl acetate. Fortified pond water recoveries were excellent ranging from 105.40 to 101.94% at the 1.03 to 0.02 mg/L levels of fortification. 2,6-difluorobenzoic acid was detected as a novel O-pentafluorobenzyl derivative reaction with pentafluorobenzyl bromide. Electron capture detector response to this derivative was excellent. An injection of 0.5 ng at attenuation 64 and a retention time of 25.63 min gave a 25% full scale deflection. The derivatization method was modified to reduce interferences from reactants (especially  $\text{Na}_2\text{CO}_3$ ), and decrease reaction time (50° for 30 min), and decrease total time of analysis by evaporation

of the completed reaction to dryness under nitrogen without losses.

Finally an examination of the diflubenzuron and 4-chloroaniline recoveries from pond water using the Freundlich equation resulted in each case in a linear relationship indicating that the decreasing recoveries with decreasing fortification level were consistent with loss due to adsorption. The  $n$  values were less than one, indicating that the isotherms may have arisen due to a minimum competition of solvent for sites of the adsorbing surface or that the solute (diflubenzuron or 4-chloroaniline) has a high affinity for the adsorbent. The improved recoveries of diflubenzuron and 4-chloroaniline from filtered pond water confirmed the existence of adsorptive immobilization of diflubenzuron and 4-chloroaniline residues by pond water particulate matter, especially organic matter.

## VI. APPENDIXES

## (a) Mass Spectra

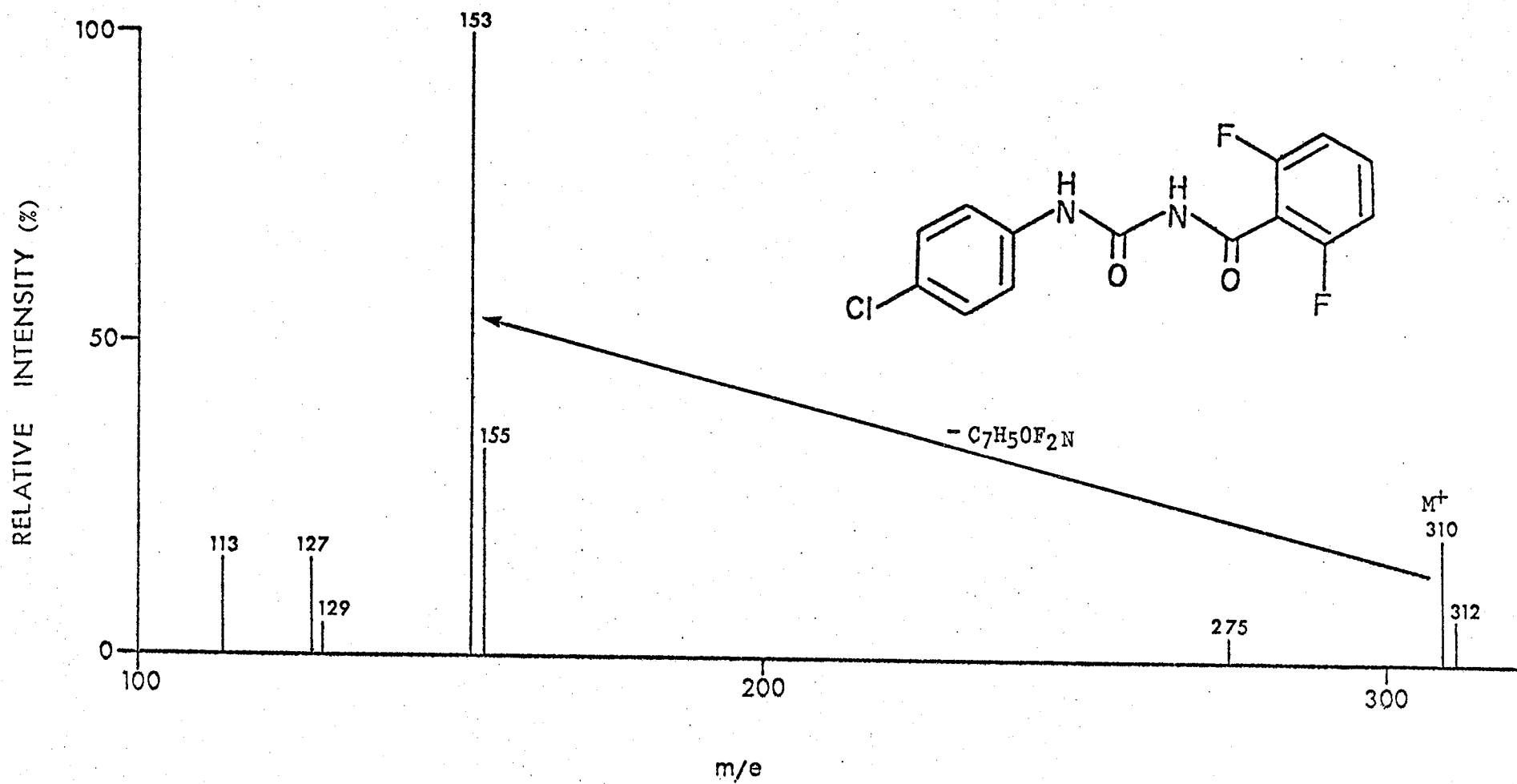


Figure 1A. Mass spectra of diflubenzuron.



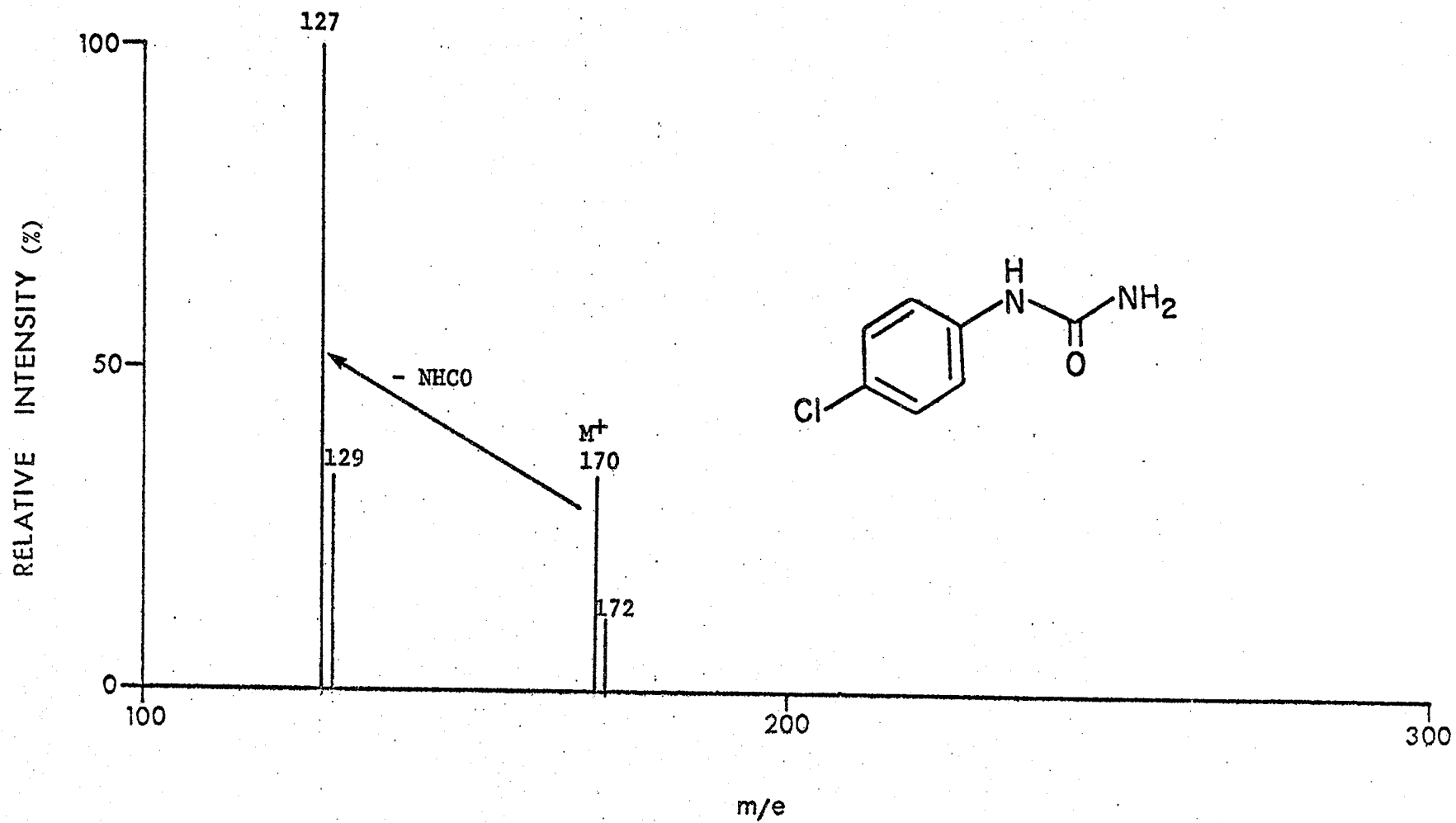


Figure 2A. Mass spectra of 4-chlorophenylurea

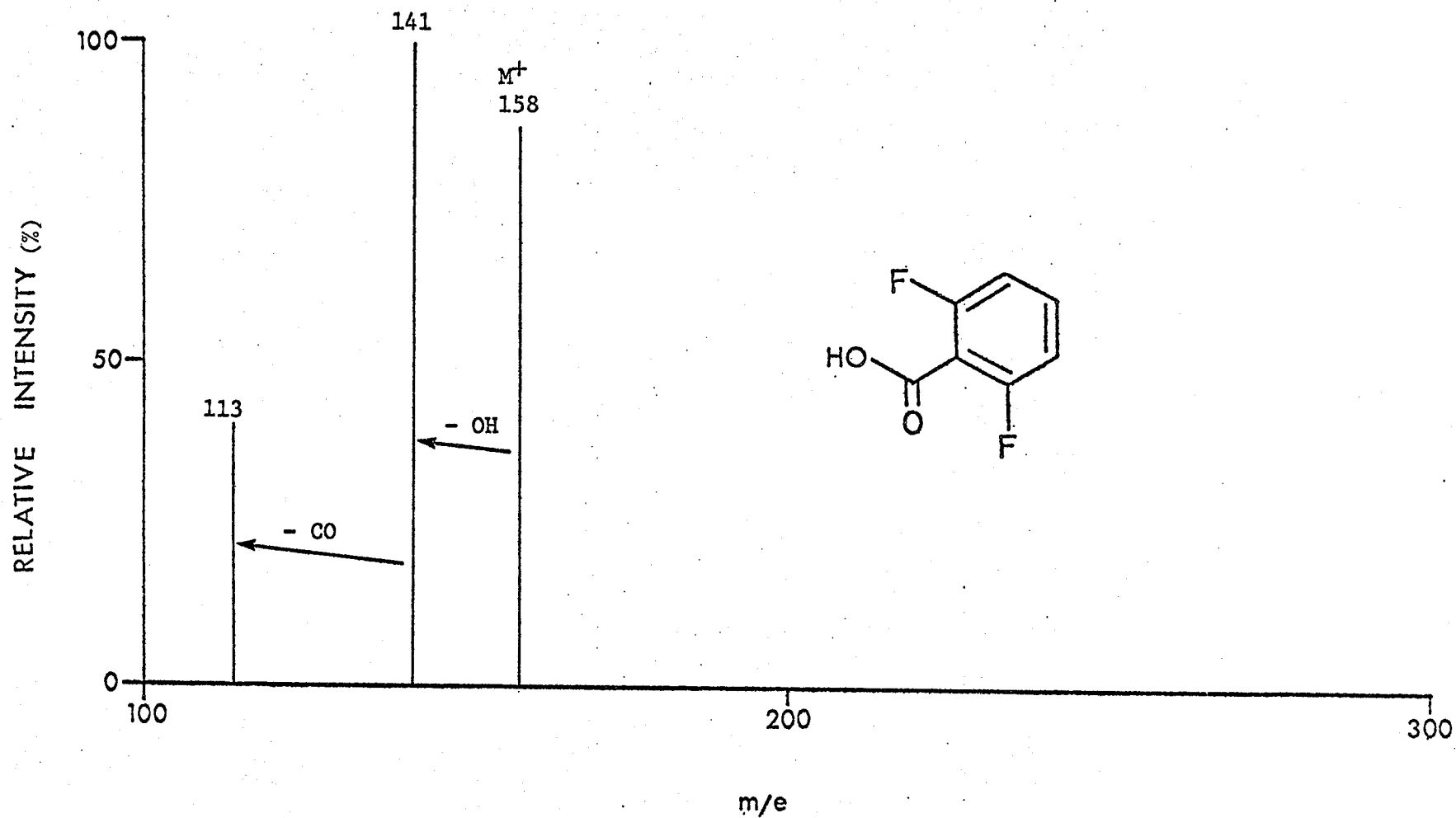


Figure 3A. Mass spectra of 2,6-difluorobenzoic acid.

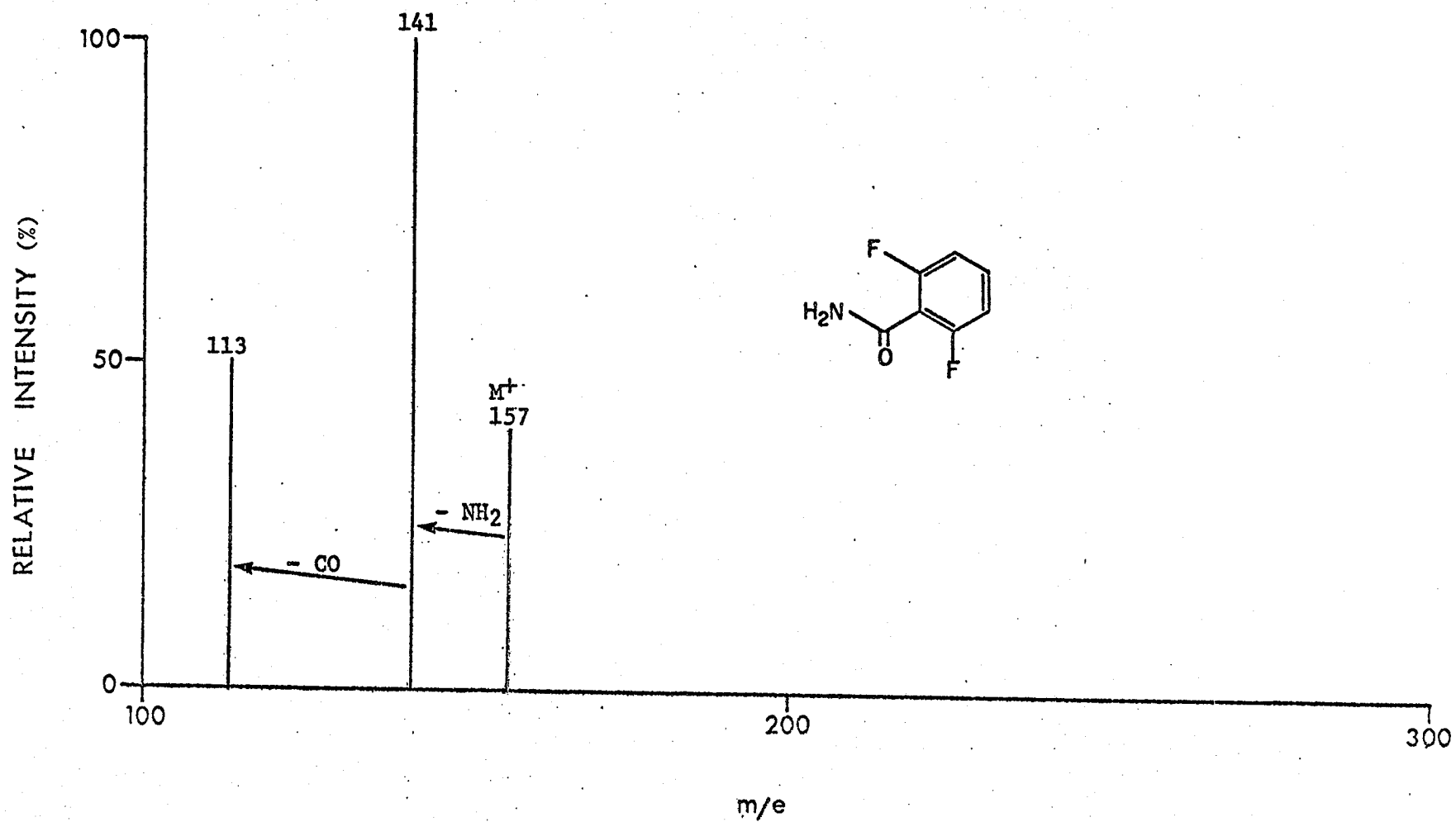


Figure 4A. Mass spectra of 2,6-difluorobenzamide.

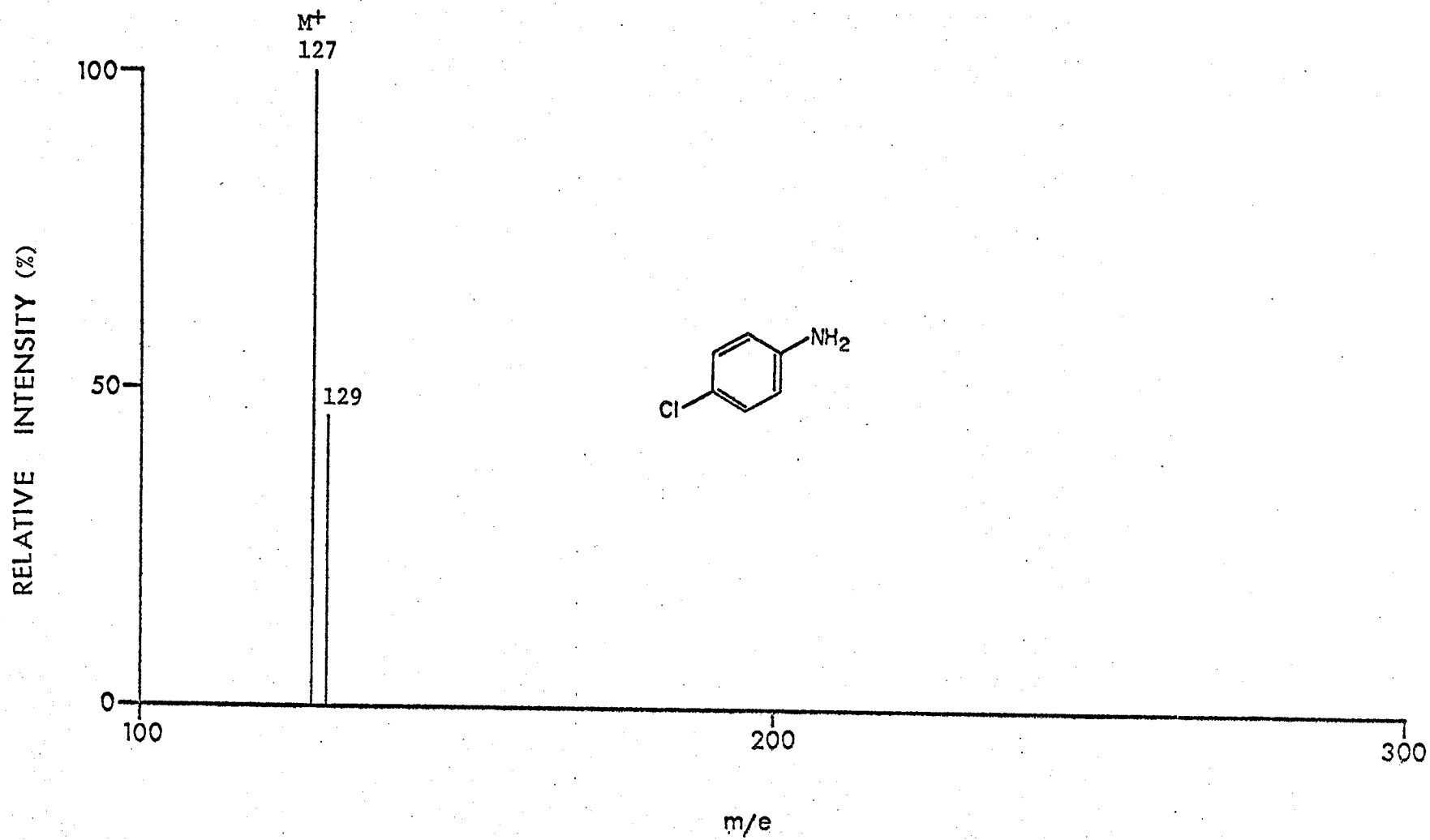


Figure 5A. Mass spectra of 4-chloroaniline.

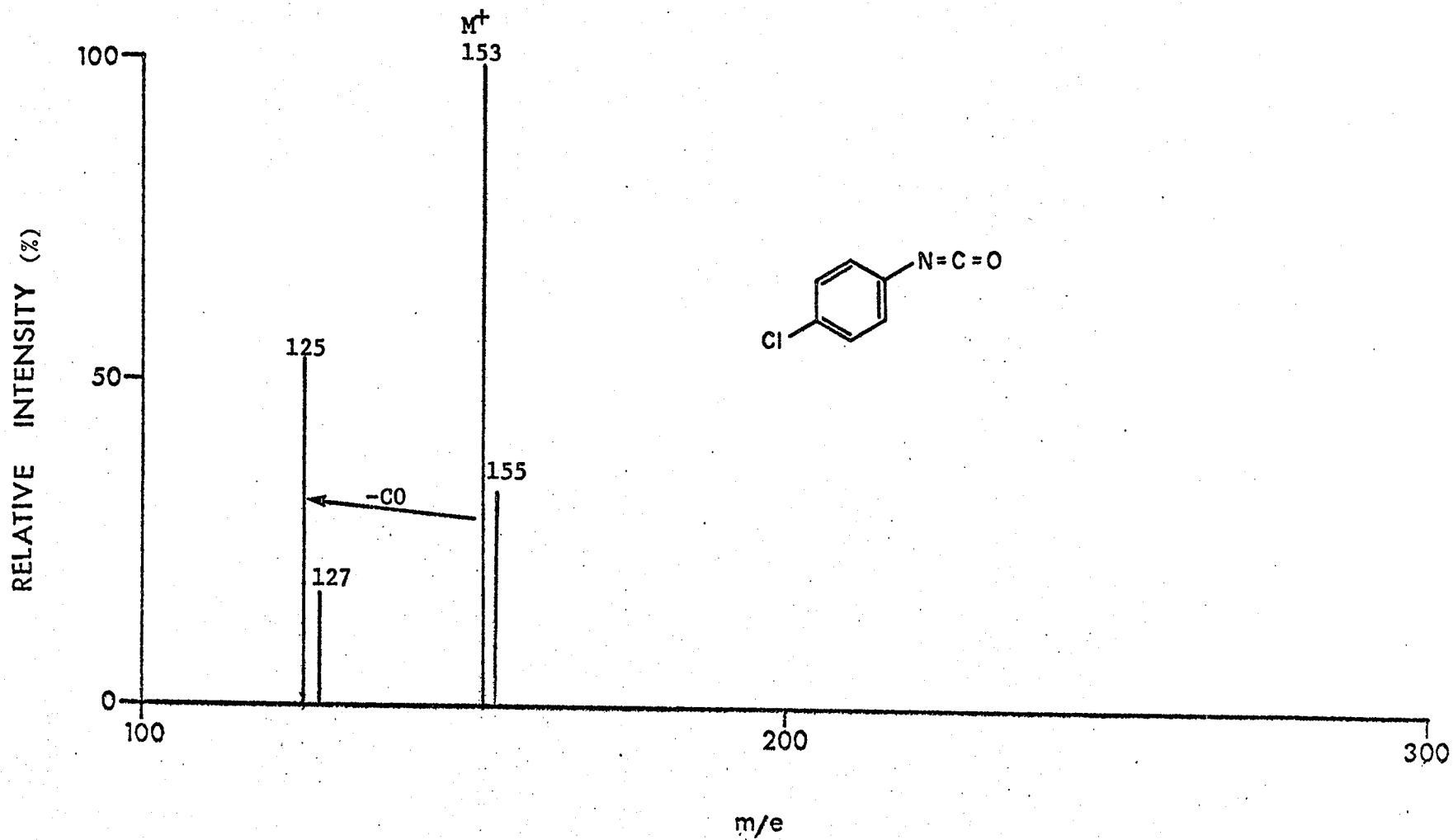


Figure 6A. Mass spectra of 4-chlorophenylisocyanate.

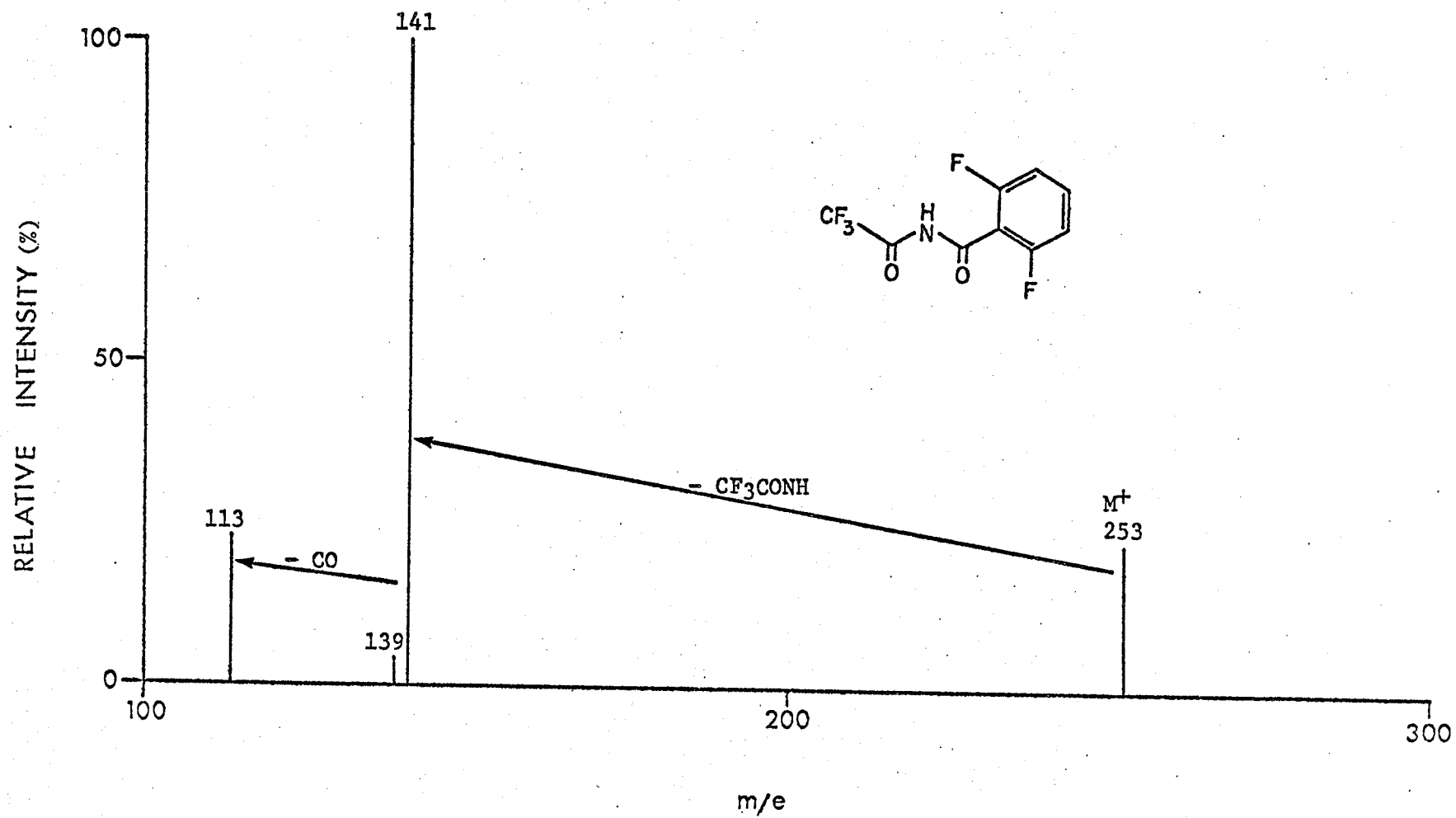


Figure 7A. Mass spectra of N-trifluoroacetyl-2,6-difluorobenzamide.

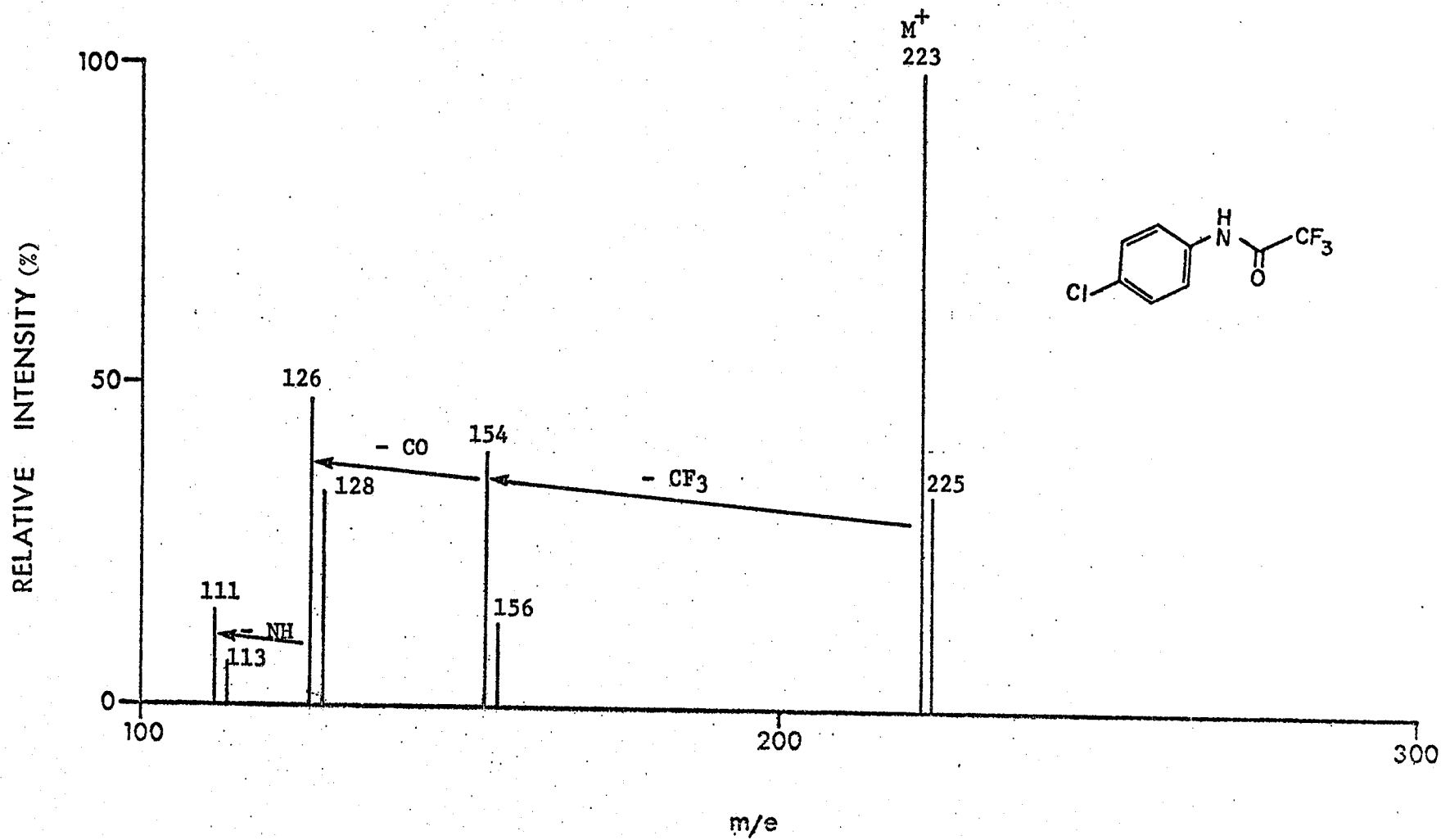


Figure 8A. Mass spectra of N-trifluoroacetyl-4-chloroaniline.

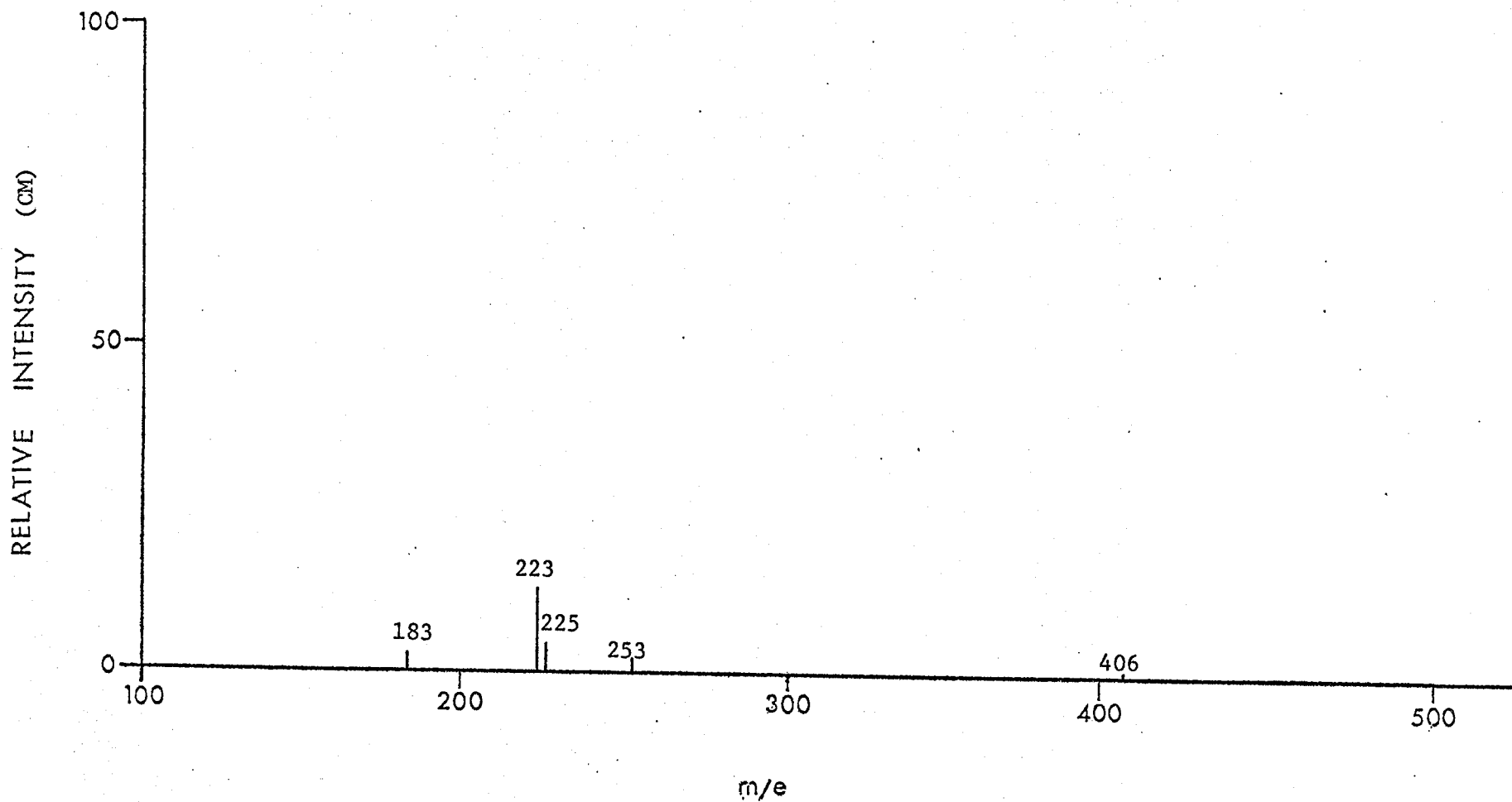


Figure 9A. Mass spectra of the reaction products of diflubenzuron with trifluoroacetic anhydride, DIP-MS (Dupont - 70ev).



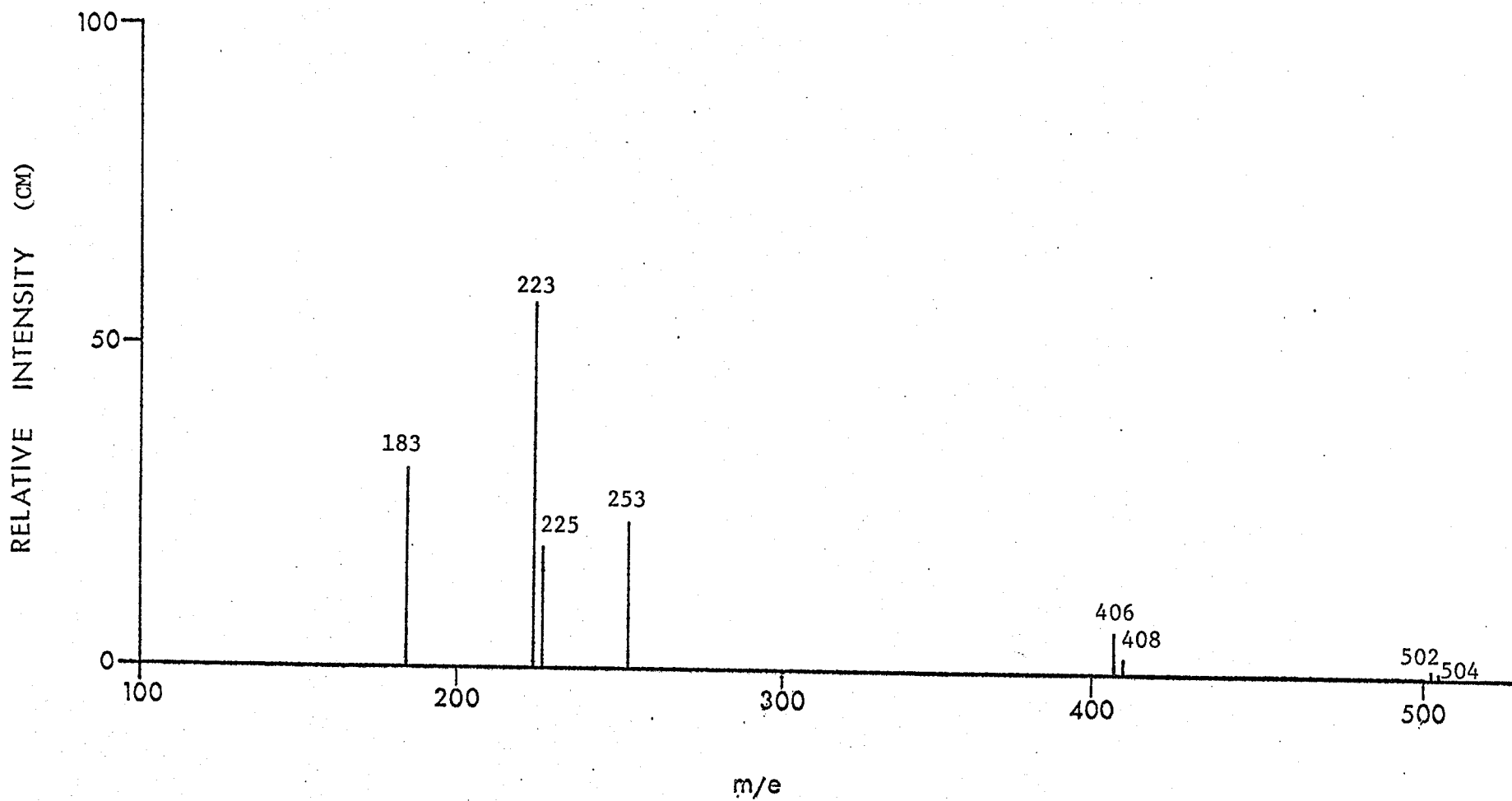


Figure 10A Mass spectra of the reaction products of diflubenzuron with trifluoroacetic anhydride, DIP-MS (Finnigan - 70ev).

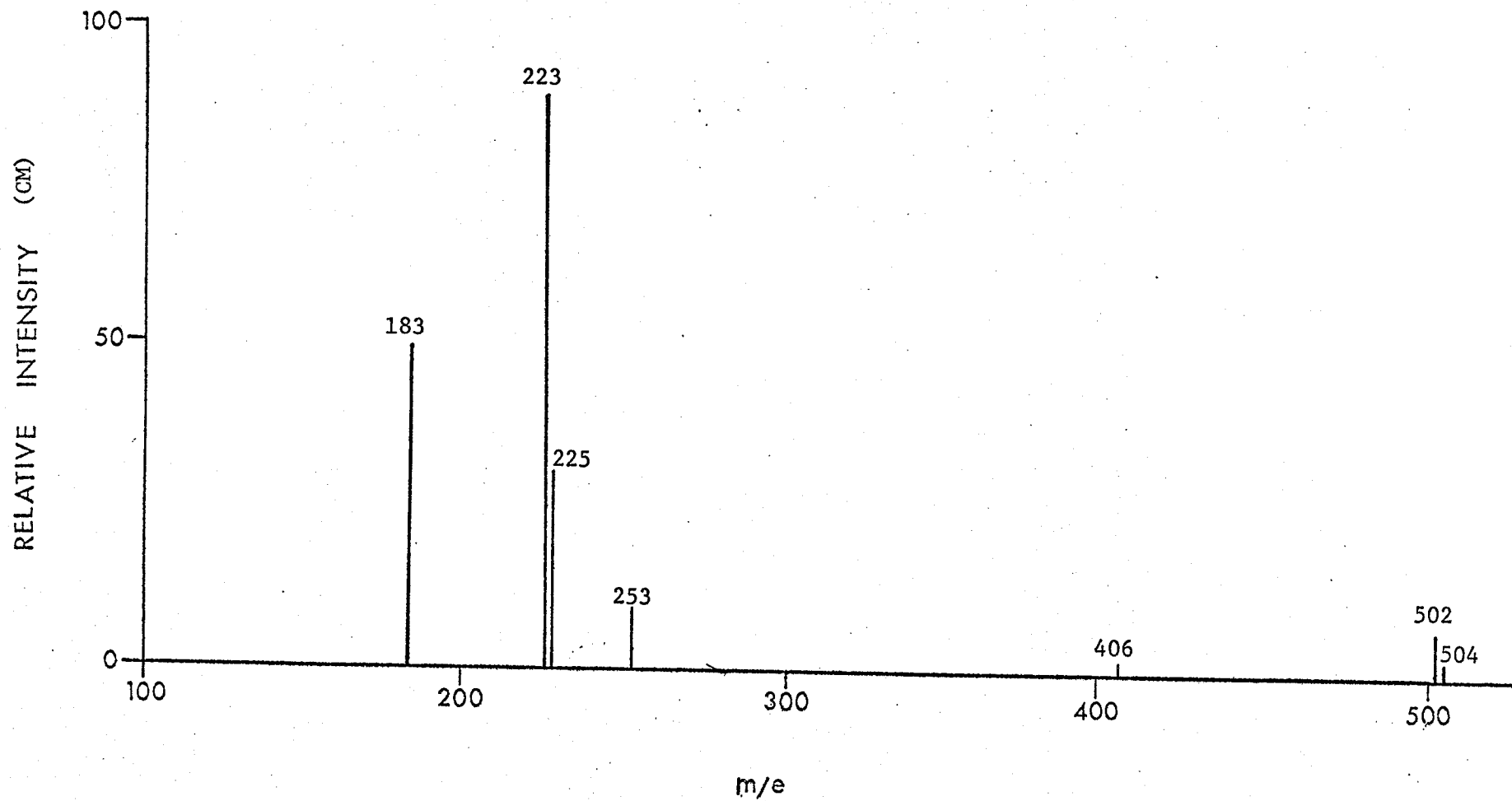


Figure 11A. Mass spectra of reaction products of diflubenzuron with trifluoroacetic anhydride, DIP-MS (Finnigan - 7ev).

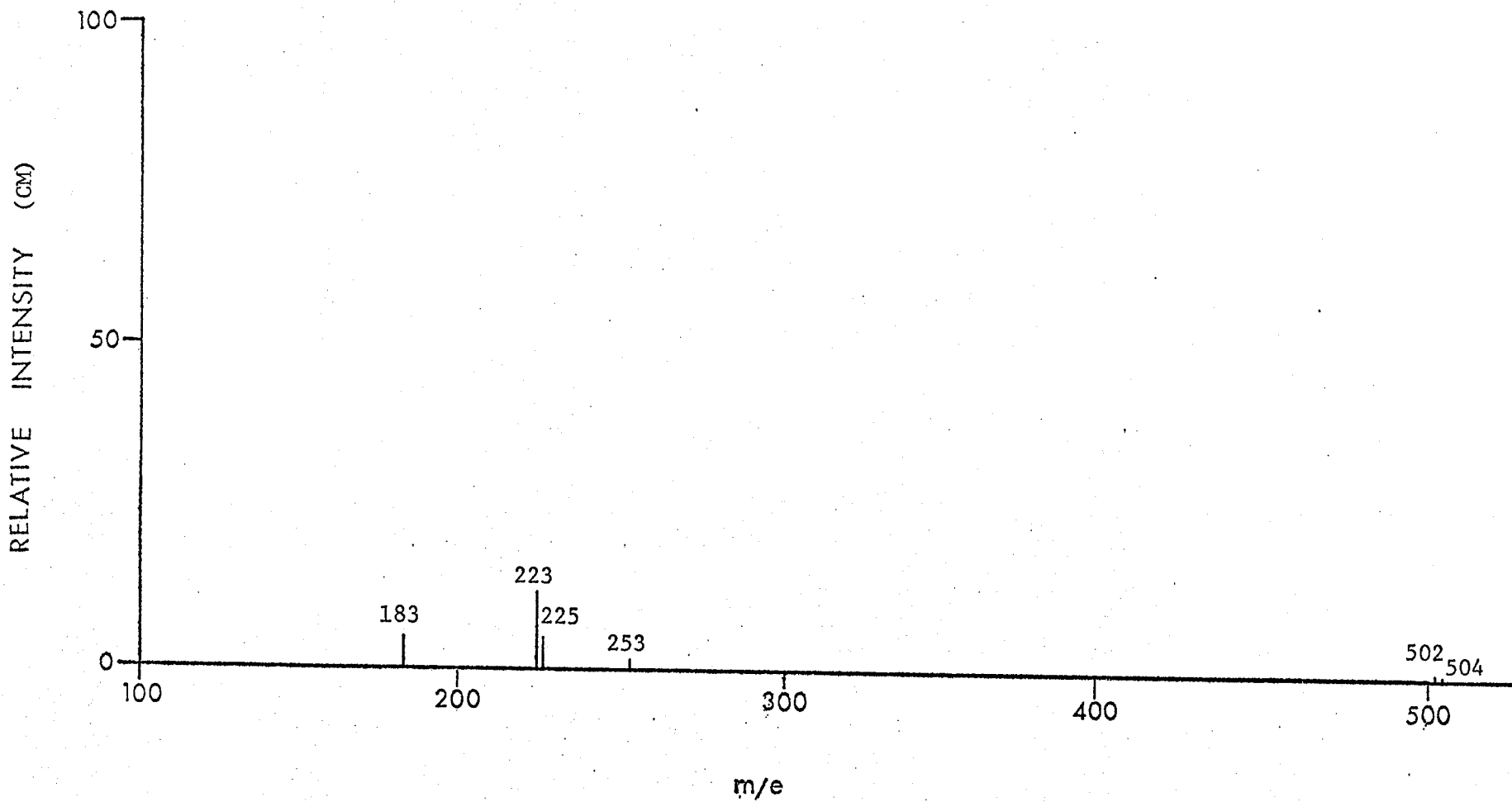


Figure 12A. Mass spectra of reaction products of diflubenzuron with trifluoroacetic anhydride, DIP-MS (Finnigan - 5ev).

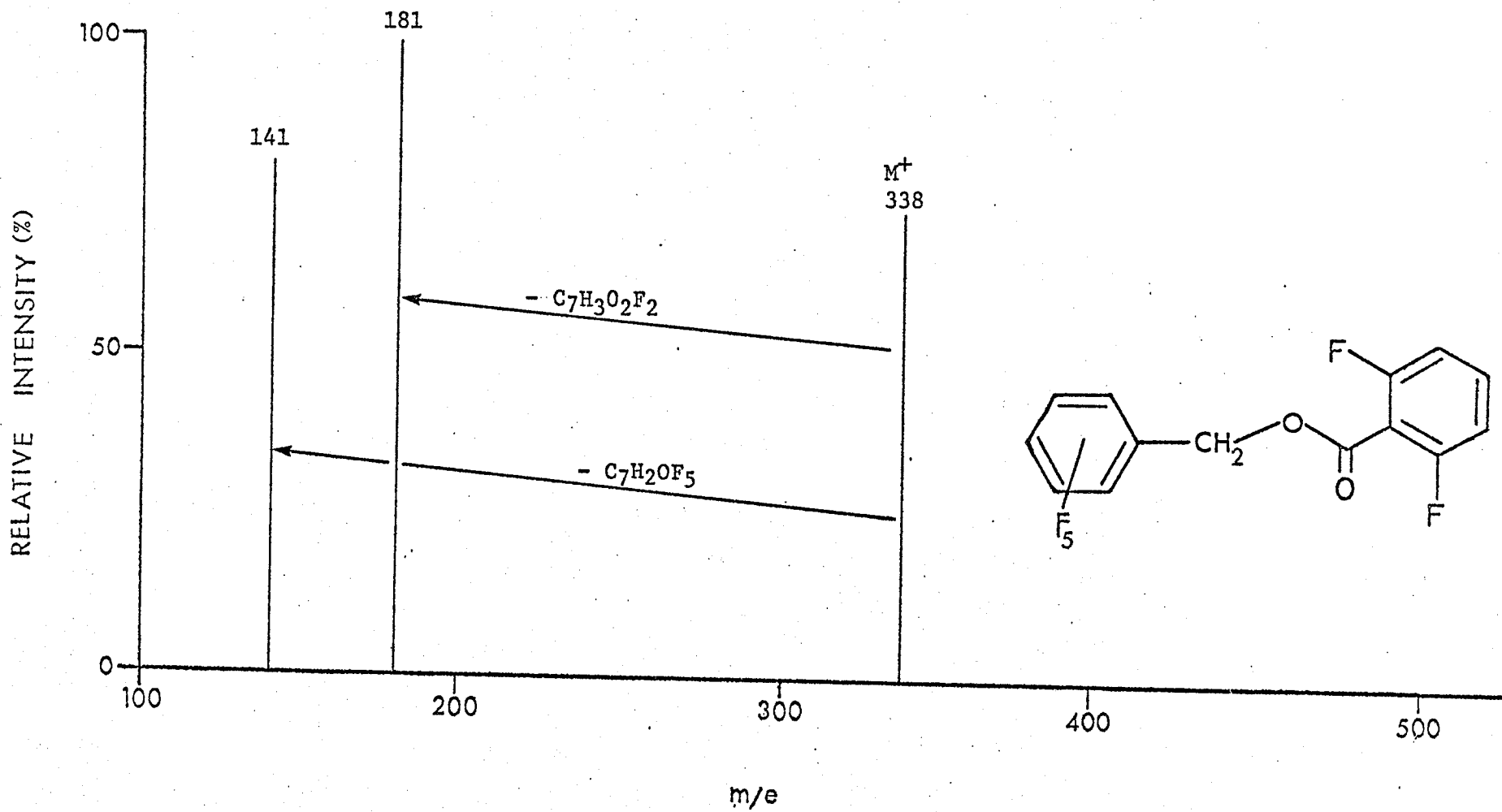


Figure 13A. Mass spectra of 0-pentafluorobenzyl-2,6-difluorobenzoic acid.

## VI. APPENDIXES

(b) Initial Attempts to Chromatograph Trifluoroacetyl Derivatives of Diflubenzuron and its Metabolites by EC-GLC.

Diflubenzuron and its metabolites (4-chloroaniline, 4-chlorophenylurea and 2,6-difluorobenzamide), except 2,6-difluorobenzoic acid appeared to react with trifluoroacetic anhydride to produce a peak(s) detectable using an electron-capture detector coupled to a gas-liquid chromatograph (EC-GLC). Initially, the column previously used for diflubenzuron (Worobey and Webster, 1977) i.e., 5% OV-101 showed 2 peaks for diflubenzuron and one peak each for the metabolites. However, the earlier peak for diflubenzuron corresponded in retention time to that of the trifluoroacetyl derivative of 2,6-difluorobenzamide and the latter peak to that of 4-chloroaniline.

In addition the peaks for 4-chloroaniline and 4-chlorophenylurea had identical retention times. Changing the column temperature and/or flow rate had no effect on separating those peaks with identical retention times.

Therefore, the following columns were investigated and chosen on the basis of different polarities of the liquid phases: 3% OV-225, 3% OV-17, 3% Silar 5CP, 5% Reoplex 400, and 4% SE-30/6% QF-1/0.2% Versamid 900. None of these columns or variations in their column temperature and/or flow rates effected a separation.

In addition the use of 4% SE-30/6% QF-1 on 130/140 mesh versus 80/100 mesh support had no effect in separating those peaks chromatographing simultaneously. Thus it appeared that these peaks were inseparable because they were the same compounds in each case. This was later confirmed by GLC-MS.

Therefore, several additional derivatizing reagents were investigated. These reagents are tabulated along with a summary of the results as shown in the following Table.

Table 1A. Reaction of diflubenzuron and its major metabolites with other derivatization reagents.

| Derivatization*<br>reagent                                 | Number of peaks seen via EC-GLC (5% OV-101) |                         |                      |                            |                               |
|--|---|-------------------------|----------------------|----------------------------|-------------------------------|
|  | diflubenzuron                               | 4-chloro-<br>phenylurea | 4-chloro-<br>aniline | 2,6-difluoro-<br>benzamide | 2,6-difluoro-<br>benzoic acid |
| TMAH   |   |                         |                      |                            |                               |
| Trimethyl-<br>anilinium hydroxide<br>(methelute)           | -   | -                       | N.R.                 | -                          | N.R.                          |
| Magic methyl<br>FSO <sub>2</sub> OCH <sub>3</sub>          | -   | -                       | N.R.                 | -                          | N.R.                          |
| Heptafluorobutyric<br>anhydride                            | 2   | 1                       | 1                    | 1 (minor)                  | -                             |
| Pentafluorobenzyl-<br>chloro formate                       | -   | -                       | -                    | I                          | I                             |
| NaH, CH <sub>3</sub> I, DMSO                               | I   | I                       | -                    | -                          | -                             |
| Alkyl-8,<br>DMF-dimethylacetal                             | N.R.  | N.R.                    | -                    | -                          | N.R.                          |
| BSTFA,<br>N,O-bis (trimethyl-<br>silyl)-trifluoroacetamide | N.R.  | N.R.                    | N.R.                 | N.R.                       | N.R.                          |

N.R. = no reaction (no product peak by EC-GLC)

I = interferences

\* = variations in reaction time, temperatures, solvents and/or use of catalysts (triethylamine and/or pyridine) were investigated.

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