Quantitative Confirmation of Ethalfluralin and Trifluralin in Soil Extracts by Negative Chemical Ionization Mass Spectrometry

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Negative chemical ionization mass spectrometry was used to quantitatively measure ethalfluralin and trifluralin in soil sampled 199 and 555 days after treatment. Concentrations measured by negative chemical ionization mass spectrometry and by a gas chromatograph with an electron capture detector were not significantly different ($P > 0.05$). With negative chemical ionization mass spectrometry, it was possible to measure and confirm concentrations of trifluralin and ethalfluralin in soil down to levels below those that are toxic to plants. The technique can thus be used to monitor "carry-over" levels as related to phytotoxicity to subsequent crop plantings.

Trifluralin is frequently used in Canada and throughout the world whereas ethalfluralin is a similar, less persistent chemical that was recently registered for use in the United States (Elanco, Division of Eli Lilly Canada, Inc., Edmonton, Alberta, personal communication). In western Canada trifluralin is persistent and can damage sensitive crops and control weeds 1 year after application (Moyer and Hamman, 1980).

Trifluralin [α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine] and ethalfluralin [*N*-ethyl-*N*-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl)benzenamine] are dinitroaniline herbicides with $-CF_3$ groups attached to aromatic rings, giving them a strong affinity for electrons. Trifluralin concentrations are normally measured with a gas chromatograph using an electron capture detector (GC-ECD) (Biros and Cummings, 1973; Smith, 1981). The electron capture detector is highly sensitive to halides and gives little response to hydrocarbons other than conjugated carbonyls (Zweig and Sherma, 1972). Residue levels are normally confirmed by using thin-layer chromatography, partition coefficients, or GC fraction collection followed by IR identification (Biros and Cummings, 1970). Mass spectrometry employing electron impact ionization techniques is used to confirm the structures of microgram quantities of pesticide residues (Biros, 1971). Mass spectrometry employing negative chemical ionization techniques (GC–MS-NCI) gives good spectra for compounds that give positive electron capture response (Dougherty and Piotrowski, 1976) and will detect nanogram quantities of pesticides without responding to most interfering biomolecules (Kuehl et al., 1980). The ability of a GC–MS-NCI to quantitatively measure organic compounds that have a positive electron affinity (Hunt et al., 1976) offers a rapid alternative method for qualitative and quantitative confirmation of dinitroaniline herbicides.

The purpose of this experiment was to demonstrate the use of the GC–MS-NCI in qualitative and quantitative confirmation of trifluralin and ethalfluralin concentrations in soil extracts.

MATERIALS AND METHODS

Trifluralin (400 g/L emulsifiable concentrate) and ethalfluralin (300 g/L emulsifiable concentrate) were applied to separate plots, May 10, 1979, of a randomized complete block experiment in four replicates, at the recommended rate of 1.0 and 1.5 kg/ha with a plot sprayer. Individual plots were 2.5 × 5 m, and the herbicides were incorporated, on May 10, 1979, by discing twice at a depth of 7.5 cm. Assuming the bulk density of the soil in the 0–15-cm layer was 1.3 g/cm^3, applications of 1.0 and 1.5 kg/ha would correspond to 500 and 750 ng of herbicide/g of oven-dried soil. The field plots were on clay loam soil with 30% clay, 45% sand, 25% silt, and 2% organic matter. A tube, 5 cm i.d. by 15 cm long, was used to take soil cores to a 15-cm depth. Five cores were taken randomly from each plot, bulked, and stored at −20 °C for analysis. Before extraction the soil was passed through a 3-mm sieve and mixed thoroughly. The soil was extracted with 10% aqueous acetonitrile, and trifluralin and ethalfluralin were partitioned into hexane (Smith, 1981). Technical-grade ethalfluralin and trifluralin, 97.5 and 98.9% purity, were used to prepare separate standards for each herbicide with 0.1, 1, 10, and 50 pg of herbicide/μL in hexane. Sufficient 3,5-dinitrobenzotrifluoride (3,5-DBT) was added as an internal standard to all extracts and standards to produce final concentrations of 50 pg/μL.

Soil was fortified at 5 and 225 ng/g with trifluralin and ethalfluralin and extracted 3 days after fortification to determine the level of recovery obtained with the extraction method.

The extracts and standards were analyzed on the GC-ECD (Perkin-Elmer Sigma III) using a 2.4 m × 2 mm i.d. glass column packed with 2.5% OV-17 on Chromosorb W HP. The temperatures of the injector, detector, and column were 250, 250, and 180 °C. The retention times were 52, 142, and 145 s for 3,5-DBT, ethalfluralin, and trifluralin, respectively. Peak areas were measured with a Spectra-Physics minigrator (Model 23000-011) set to integrate peaks that are superimposed on a trailing solvent peak. The ratios of peak areas for ethalfluralin and trifluralin standards to peak areas for the 3,5-DBT internal standard were calculated, and linear regression equations for trifluralin and ethalfluralin concentration vs. the ratios were developed. For extracts from fortified samples and the field samples, the ratios of ethalfluralin and trifluralin peak area to 3,5-DBT peak area and the regression equations were used to determine concentrations.

To confirm the concentrations, all samples were analyzed on a quadrupole GC–MS-NCI (Hewlett-Packard Model 5985B). The samples were injected by splitless mode into a 20-m glass capillary column coated with OV-275. The injector temperature was 225 °C, and the initial column temperature was 50 °C for 1 min and increased from 50 to 140 °C at 30 °C/min and from 140 to 180 °C at 4 °C/min. The flow rate for the carrier gas, He, was 30 cm/s. The mass spectrometer was operated in the negative chemical ionization mode with methane reactant gas. Transfer lines were at 210 °C, and the ion source was at 100 °C. A pressure of (6–8) × 10^{-5} torr was maintained. Initially 50 pg/μL standards of trifluralin, ethalfluralin,

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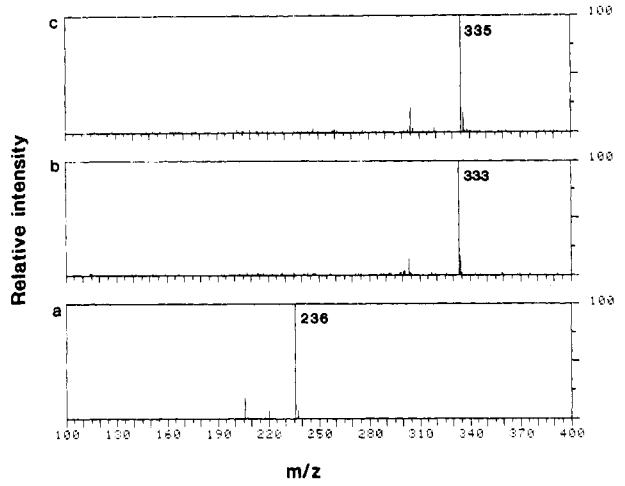

Figure 1. Negative chemical ionization mass spectra of (a) 3,5-DBT, (b) ethalfluralin, and (c) trifluralin.

and 3,5-DBT were run in the scanning mode (m/z 100–400) to determine retention times, sensitivities, and the NCI spectra of these compounds. For quantitative results, the selected ion mode was used for standards and extracts. The retention times were 6.0, 6.4, and 6.2 min for 3,5-DBT, ethalfluralin, and trifluralin, respectively. The GC–MS-NCI responses for standards and extracts were used to calculate concentrations of trifluralin and ethalfluralin.

The concentrations of trifluralin and ethalfluralin, measured in the field samples with the GC–MS-NCI and GC-ECD, were compared by analyzing the data as a factorial with replicates, methods of analysis (GC–MS-NCI and GC-ECD), dates of sampling (199 and 555 days after treatment), and rates of applications (1.0 and 1.5 kg/ha) as factors. Similarly, recovery data for fortified samples were compared in an analysis of variance with replicates, methods of analysis (GC–MS-NCI and GC-ECD), and fortification level (5 and 225 ng/g) as factors.

RESULTS AND DISCUSSION

The NCI mass spectra of 3,5-DBT, ethalfluralin, and trifluralin exhibited predominantly the M^- ions at m/z 236, 333, and 335, respectively (Figure 1). For quantitative analysis, mass chromatograms were produced when the selected ion monitors were set at m/z 236, 333, and 335 for these compounds (Figure 2).

The correlation coefficients for the linear relationship between ethalfluralin and trifluralin concentrations and the ratio of peak areas, determined by the GC–MS-NCI, for ethalfluralin and trifluralin to 3,5-DBT were >0.990 and 0.990, respectively.

With the GC-ECD, correlation coefficients for the same linear relationships were >0.990 for both ethalfluralin and trifluralin. These correlation coefficients indicate there is a good linear response by both the GC–MS–NCI and GC-ECD to ethalfluralin and trifluralin over the concentration ranges required for analysis of the field soil extracts.

Recoveries of trifluralin and ethalfluralin measured with the two instruments were not significantly different ($P > 0.05$) (Table I). The variability in percent recovery was much less for the GC–MS-NCI, particularly at the low levels of fortification.

The lower limits of detection for trifluralin and ethalfluralin, using the rule of thumb that the lower limit of detection is twice the signal obtained from a blank sample (Byast et al., 1977), are 0.25 and 1 ng/g for the GC–MS-NCI and the GC-ECD.

Concentrations of trifluralin and ethalfluralin, in extracts from field samples, measured by the GC-ECD and GC–MS-NCI were not different ($P > 0.05$) (Table II). As the GC–MS-NCI was set to measure only the M^- ions of 3,5-DBT, trifluralin, and ethalfluralin and the retention times were correct for these herbicides, the GC–MS-NCI has provided strong evidence that concentrations measured with the GC-ECD represent pure trifluralin that was not enhanced by metabolites or other contaminants extracted with aqueous acetonitrile. The extracts were from soil fortified 3 days prior to extraction and from field soils that had 555 days for metabolites to form prior to extraction. Concentrations after 555 days approach the detection limit of the GC-ECD and are below levels that cause reductions in the growth of green foxtail [*Setaria viridis* (L.)], a grass that is very susceptible to trifluralin and ethalfluralin

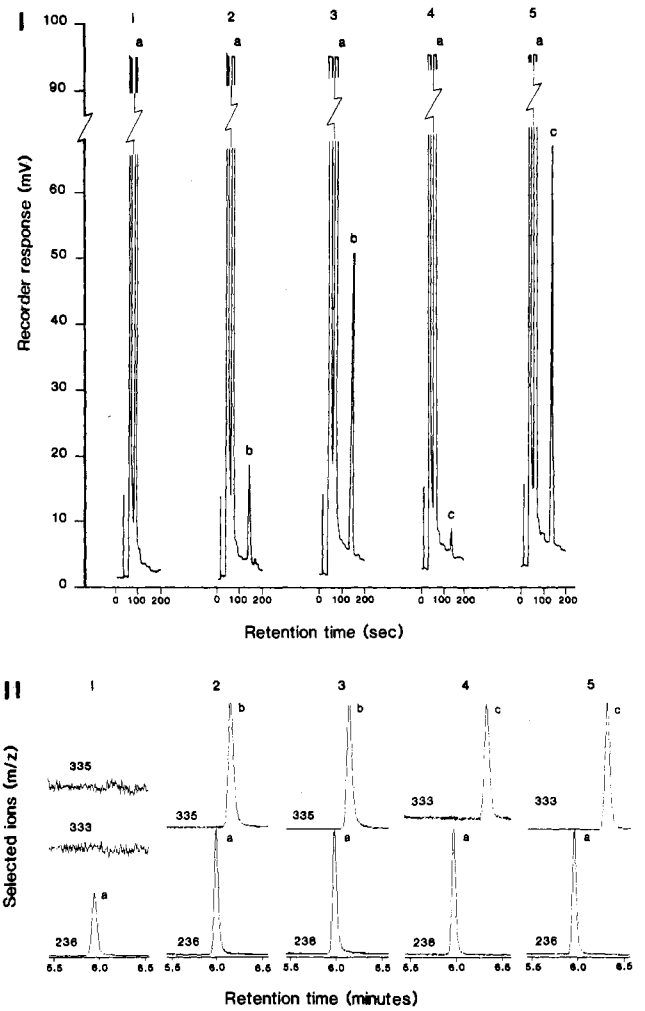

Figure 2. Typical chromatograms for (I) GC-ECD at attenuation 32 and (II) GC–MS-NCI with peaks set to 100% scale. Chromatograms are (1) untreated field sample, (2) 1.5 kg/ha rate of trifluralin 555 days after treatment, (3) soil fortified with 225 ng/g trifluralin, (4) 1.5 kg/ha rate of ethalfluralin 555 days after treatment, and (5) soil fortified with 225 ng/g ethalfluralin. Peaks are (a) 3,5-DBT, (b) trifluralin, and (c) ethalfluralin.

Table I. Recovery of Ethalfluralin and Trifluralin

fortification level, ng/g	% recovery ± SEM[a]	
	GC–MS-NCI	GC-ECD
	Ethalfluralin	
5	93 ± 1	118 ± 12
225	105 ± 4	105 ± 5
	Trifluralin	
5	93 ± 2	97 ± 9
225	96 ± 1	106 ± 2

[a] SEM = standard error of the mean.

Table II. Concentration of Ethalfluralin and Trifluralin Measured with a GC–MS-NCI and a GC-ECD[a]

rate, kg/ha	days after treatment	concentration, ng/g of oven-dry soil GC–MS-NCI	GC-ECD
		Ethalfluralin	
1.0	199	46	50
1.5	199	124	129
1.0	555	12	12
1.5	555	16	23
standard error of mean for methods of analysis = 2.6			
		Trifluralin	
1.0	199	122	126
1.5	199	206	192
1.0	555	57	65
1.5	555	89	85
standard error of mean for methods of analysis = 4.8			

[a] Differences in concentration of trifluralin and ethalfluralin due to rates and sampling dates were significant and differences between GC–MS-NCI and GC-ECD were not significant ($P = 0.05$) by analysis of variance.

(Lethbridge Research Station, unpublished data). Thus, the GC–MS-NCI can be used to confirm levels of ethalfluralin and trifluralin residues that are toxic to plants.

The ability to analyze trifluralin and ethalfluralin with the GC–MS-NCI indicates that this system has potential for rapidly confirming structures and concentrations of several pesticides with a positive electron affinity in soil extracts.

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Registry No. Ethalfluralin, 55283-68-6; trifluralin, 1582-09-8.

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Herbicidal Mode of Action on Chlorophyll Formation

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Oxadiazon [2-*tert*-butyl-4-(2,4-dichloro-5-isopropoxyphenyl)-Δ^2-1,3,4-oxadiazolin-5-one], MK-616 [*N*-(4-chlorophenyl)-3,4,5,6-tetrahydrophthalimide], and DTP [1,3-dimethyl-4-(2,4-dichlorobenzoyl)-5-hydroxypyrazole] exert phytotoxic activity by interfering with chlorophyll biosynthesis as a primary mode of action. When *Scenedesmus* is grown in the dark with these herbicides present, they interfere with chlorophyll biosynthesis, whereas carotene biosynthesis is unaffected. In addition, the content of plastidic cytochrome *c*-553 decreases. Peroxidative activity of oxadiazon (assayed by ethane evolution or degradation of ^{53}S-prelabeled sulfolipid) is very low at a concentration of 1 μM oxadiazon, which is the I_{50} value for inhibition of chlorophyll formation. With 10 μM oxadiazon present, peroxidative properties are evident. The fluorescence-induction kinetics of *Scenedesmus* grown with 1 μM oxadiazon present are different from those obtained with norflurazon [4-chloro-5-(methylamino)-2-[3-(trifluoromethyl)phenyl]pyridazin-3(2*H*)one] or the peroxidative herbicide oxyfluorfen [2-chloro-4-(trifluoromethyl)phenyl]3-ethoxy-4-nitrophenyl ether]. In contrast to the latter two, the signal shape is preserved with oxadiazon, while the signal height diminishes in oxadiazon-treated cells due to a decrease of the chlorophyll content.

Little information is available on the mode of action of many commercial herbicides. One of these compounds is oxadiazon, which is used for weed control in rice and soybean as well as in orchards and vineyards (Burgaud et al., 1969; Ambrosi and Desmoras, 1973). In a recent publication, evidence was presented that oxadiazon alters the pigment composition of the chloroplast (Sandmann and Böger, 1983a). So-called "bleaching" herbicides can exert their influence by either inhibiting biosynthesis of (1) chlorophyll or (2) carotenoids and (3) by causing destruction of pigments already formed (Sandmann and Böger, 1982a).

In this paper, data are presented indicating that a primary target of oxadiazon is the biosynthetic pathway of chlorophylls. In short-term experiments, peroxidative properties of this herbicide were negligible when using concentrations of 1 μM. In addition, phytotoxic activities of substituted hydroxypyrazoles (Moon et al., 1977) and

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