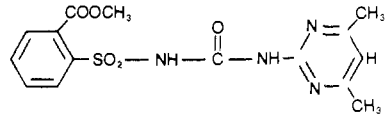
Analysis of the Herbicide Sulfometuron Methyl in Fish and Green Plants by Liquid Chromatography

Edward W. Zahnow

The method developed for the analysis of sulfometuron methyl, methyl 2-[[[[(4,6-dimethyl-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]benzoate, in soil and water has been extended to the analysis of fish and green plants. By using suitable extraction, cleanup, and isolation procedures, sulfometuron methyl can be measured at levels as low as 5 ng/g (5 ppb) in fish and 10 ng/g (10 ppb) in green plants.

The active ingredient in Du Pont Oust herbicide, sulfometuron methyl, is a sulfonylurea of the structure



Sulfometuron Methyl

Oust has a wide variety of uses and application rates (Du Pont, 1984). Since the decomposition rate of sulfometuron methyl is very rapid, the method used for environmental samples (soil, water, fish, green plants) developed to measure sulfometuron methyl must reliably measure very small quantities that might remain. The method reported for the analysis of soil and water (Zahnow, 1985) avoids derivatization of sulfometuron methyl, and the operating conditions are sufficiently mild that decomposition is avoided.

The sensitivity requirements for sulfometuron methyl in fish and green plants are somewhat greater than normally encountered, and previous work in this laboratory has shown that background response is diminished and the sulfometuron methyl response is enhanced if a photoconductivity detector is used in place of a UV absorbance detector. Similar observations have been reported by Bush et al. (1984), Jasinski (1984), Walters (1983), and Walters and Gilvydis (1983) for other compounds.

EXPERIMENTAL SECTION

Preliminary Treatment. The entire sample, which should be in frozen condition, was homogenized with dry ice in a Waring or Hobart blender (depending on sample size) until it reached a powder consistency. A large fish must be sectioned before blending, and the individual batches have to be thoroughly mixed together. The homogenized sample should be stored in a freezer until needed.

Extraction Procedure. A 20-g sample (10-g sample of green plants) of fish was mixed with 100 mL of acetonitrile in a 250-mL glass centrifuge bottle for 2 min using a Tekmar Tissumizer (shaft SDT182EN). The resulting mixture was centrifuged for 5 min at 1500 rpm, and the supernatant liquid was decanted into a 500-mL separatory funnel. The solids remaining in the bottle were extracted a second time in the same manner, and the liquid was combined with that from the first extraction.

Cleanup Procedure. The acetonitrile solution was extracted three times (twice for green plants) with 50-mL portions of hexane, shaking for 1 min. The hexane layers (upper) were separated and discarded.

The acetonitrile soluton was then transferred to a 300-mL, pear-shaped flask. After 1 mL of glacial acetic acid was added to the acetonitrile solution, the solution was concentrated to 3–5 mL on a rotary evaporator at 25–30 °C. Then, 50 mL of aqueous 0.1 M Na_2CO_3–0.1 M $NaHCO_3$ was put into the flask and mixed thoroughly. This solution was transferred to a 250-mL separatory funnel with a small (<5 mL) water wash, and it was extracted three times (twice for green plants) with 50-mL portions of hexane, shaking for 1 min. The hexane layers (upper) were separated and discarded.

The aqueous solution was drained from the separatory funnel into a 250-mL beaker, and the pH was adjusted to 3.5 by adding 10% HCl *dropwise* while measuring with a calibrated pH meter. In this pH range sulfometuron methyl, which is a weak acid, was un-ionized and could be extracted into various organic liquids. The pH adjustment had to be performed carefully since carbon dioxide was evolved, causing foaming, and, also, due to the buffered system involved, the pH changed slowly. At a very low pH there would be a danger of chemical decomposition of sulfometuron methyl, whereas if it would be too high, complete extraction could not be achieved.

The solution was then transferred into a 250-mL separatory funnel, with 5 mL of distilled water being used to rinse the beaker. The contents were then extracted three times with 50-mL portions of toluene by agitating vigor-

Table I. Recoveries of Sulfometuron Methyl from Fortified Fish[a]

species	fortification, ppb	recovery, % mean	SD	range
bass	5.0	88	6	80–94
	25	88	7	77–94
bluegill	5.0	90	8	77–100
	25	87	7	79–94
trout	5.0	87	4	84–93
	25	86	4	83–94

[a] $N = 6$ for each fish species at each fortification level.

Table II. Recoveries of Sulfometuron Methyl from Green Crops[a]

crop	fortification, ppb	recovery, % mean	SD	range
alfalfa (stems and sprouts)	10	88	4	86–95
	50	95	6	88–102
corn (leaves)	10	89	9	81–106
	50	86	5	81–92
rice (whole plants)	10	91	8	84–105
	50	88	4	80–92
wheat (forage)	10	88	14	69–106
	50	91	3	86–95

[a] $N = 6$ for each crop at each fortification level.

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ously for 1 min. Each toluene extract was separated from the aqueous phase and then combined in a 300-mL pear-shaped flask. When centrifuging was required to break an emulsion, only glass centrifuge bottles were used. The combined extracts were examined carefully to ensure that they were free of water droplets. If water droplets were present, they were removed by decanting the toluene solution from one glass container into another. To the toluene extract was added 1 mL of glacial acetic acid, and the solution was taken to dryness with a rotary evaporator at about 45 °C with a water aspirator as the vacuum source.

The flask was washed four times with 2-mL portions of methanol. Each washing was carefuly transferred with a Pasteur capillary pipet into a 150-mL beaker containing 72 mL of water that had been previously adjusted to pH 3.5 with 0.1 N HCl by a calibrated pH meter. Each methanol washing was mixed into the water thoroughly at the time of transfer.

As a final cleanup step, this solution was put through a C2 Bond Elut column (500 mg/2.8 mL, Analytichem International) that had been previously washed with 10 mL of methanol followed by 25 mL of a mixture of pH 3.5 water and methanol (9:1, v/v). Next, the column was washed with 10 mL of the 9:1 water–methanol solution which was discarded. Following this step, the column was eluted with 8 mL of ethyl acetate, and the eluant was collected in a 10-mL centrifuge tube. (Note: Solvents were pulled through the Bond Elut column by applying vacuum. The flow rate was such that discrete drops could be seen coming from the column.) When a water layer was found in the centrifuge tube, the ethyl acetate layer (upper) was removed with a Pasteur capillary pipet and placed into a 10-mL centrifuge tube. A gentle nitrogen stream was used to evaporate the ethyl acetate solution to dryness at 40 °C. The sample was stored dry in a refrigerator until it was to be analyzed.

For analysis, the sample was dissolved in Solution C (750 mL of cyclohexane, 125 mL of 2-propanol, 125 mL of methanol), with dilution to a final volume of 1 mL for fish extracts and 5 mL for green plant extracts. The entire sample was then filtered into a small vial using a Millipore Millex-SR 0.5-μm filter unit mounted on a hypodermic syringe. These filter units were discarded after use.

ANALYSIS

The HPLC procedure described (Zahnow, 1985) for soil and water was used, and working standards for analysis and fortification were prepared at suitable concentrations over the range 0.02–0.50 μg/mL.

Detector response factors are generally stable and consistent on a day-to-day basis, but occassionally large changes can occur due to variations in mobile phase composition or detector lamp condition. Consequently, frequent analysis of standards is essential.

RESULTS AND DISCUSSION

For the recovery study, three species of freshwater fish and four types of green plants were chosen. These were fortified at two levels: the detection limit and a level 5 times greater. Fortification was performed by pipetting a suitable quantity of a working standard onto the weighed sample and evaporating the solvent with a gentle stream of nitrogen. The results of the measurements are shown in Tables I and II, and from these it can be seen that recovery is not dependent on species of fish, type of green plant, or fortification level.

The chromatograms of an extract of trout and of an extract of fortified trout (5 ppb) are shown in Figure 1.

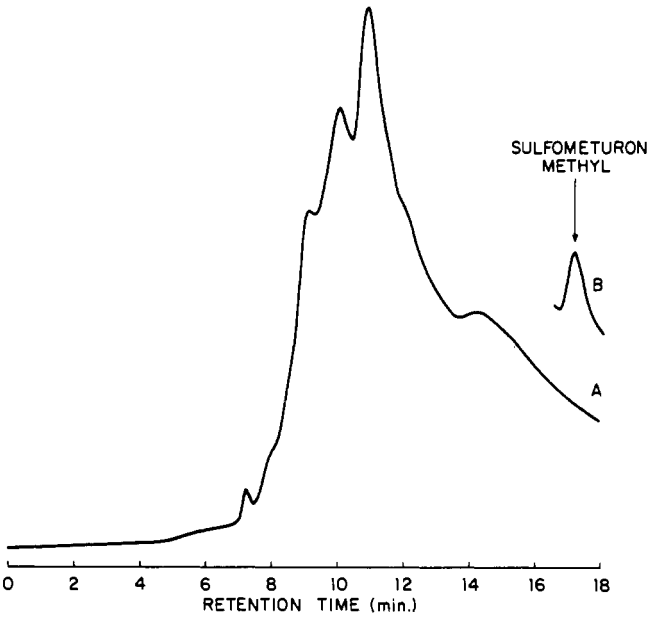

Figure 1. Chromatograms of trout extracts (detector sensitivity 1×5): (A) control; (B) 5 ppb fortification.

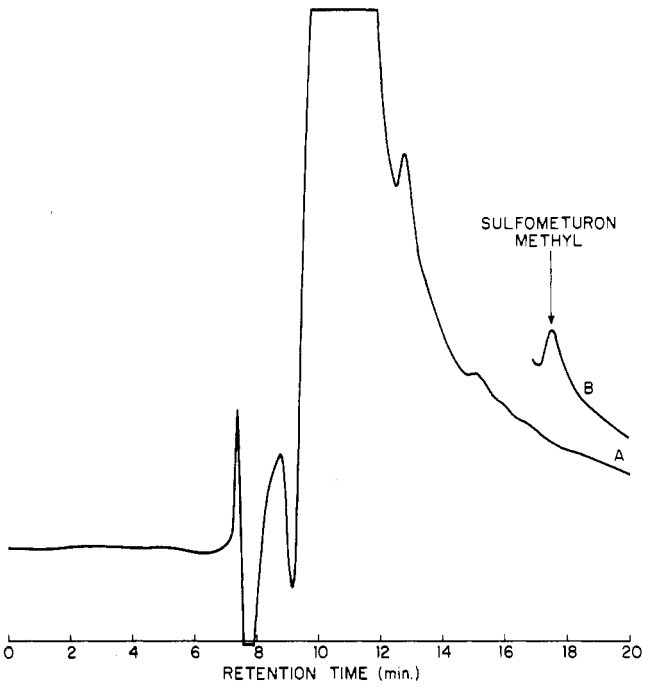

Figure 2. Chromatograms of alfalfa extracts (detector sensitivity 1×1): (A) control; (B) 10 ppb fortification.

The average recovery was 87%. Comparable chromatograms were obtained with the bass and bluegill extracts.

The chromatograms of an extract of alfalfa and of an extract of fortified alfalfa (10 ppb) are shown in Figure 2. The average recovery was 88%. Similar chromatograms were obtained with the other three green crops.

CONCLUSION

Sulfometuron methyl, which is the active ingredient in Oust herbicide, can be effectively measured in freshwater fish and green plants. The limit of detection is 5 ppb for fish and 10 ppb for green plants when a normal-phase HPLC separation is used with the highly sensitive and selective photoconductivity detector, and average recoveries are near 90%.

Registry No. Sulfometuron methyl, 74222-97-2.

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O-(α-Cyano-*m*-phenoxybenzyl) *N*-Alkyl- and *N*-Aralkylcarbamates and Related Pyrethroid-like Insecticides

Osamu Kirino[1] and John E. Casida*

Structure–activity relationships are examined for the toxicity to houseflies of pyrethroid-like carbamates, esters, and related compounds lacking a cyclopropane ring. The isosteric *tert*-butyl α-bromoacetate and *N*-*tert*-butylcarbamate are effective acid moieties with α-cyano-*m*-phenoxybenzyl, *m*-phenoxybenzyl, and other pyrethroid alcohols and the oxidase inhibitor piperonyl butoxide strongly synergizes the toxicity in each case. The esterase inhibitor phenylsaligenin cyclic phosphonate is generally more effective in synergizing the carboxylic esters than the carbamates. Substituent effects on the activity of 15 *O*-(α-cyano-*m*-phenoxybenzyl) *N*-alkylcarbamates are shown by a modified Free–Wilson method to be related to the number of branches in the alkyl group in which α branching is favorable and β and γ branching are unfavorable for the activity. *O*-(α-Cyano-*m*-phenoxybenzyl) *N*-[(*R*)-α-methylbenzyl]carbamate is much more toxic than the *S* isomer. In a series of esters, amides, and ethers, critical features for activity are both the distance between the *tert*-butyl and *m*-phenoxyphenyl groups and the nature of the central linkage providing this distance.

Structural modifications of the chrysanthemate moiety of pyrethroids establish that the isobutenyl moiety and cyclopropane ring are not absolute requirements for insecticidal activity but the *gem*-dimethyl group or an equivalent substituent is essential (Barlow et al., 1971; Berteau and Casida, 1969; Elliott and Janes, 1978; Henrick et al., 1980; Matsui and Kitahara, 1967; Ohno et al., 1974). Further simplification of the pyrethroid acid moiety led to the *tert*-butyl acetate, but it has little activity (Elliott et al., 1983), possibly due in part to rapid hydrolysis of the ester linkage. Esteratic detoxification might be minimized or circumvented by increasing the steric bulk of the acid moiety or changing the nature of the central linkage, e.g. introducing a bromine atom in the α-position of the *tert*-butyl acetate (Kirino et al., 1983), modifying the carboxylic acid ester to a carbamic acid ester linkage (Berteau and Casida, 1969), or using a central linkage other than an ester (Berteau and Casida, 1969; Bull et al., 1980; Nakatani et al., 1982). The present structure–activity study applies these approaches to *O*-(α-cyano-*m*-phenoxybenzyl) *N*-alkyl and *N*-aralkylcarbamates and related pyrethroid-like insecticides.

MATERIALS AND METHODS

Chemicals. The compounds listed in Tables I–VI were synthesized by conventional methods. Carbamates were prepared by one of three procedures: (1) reaction of an isocyanate with an alcohol in tetrahydrofuran in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene as a catalyst; (2) reaction of an amine with a chloroformate (obtained from an alcohol and phosgene in benzene in the presence of triethylamine and used without purification); (3) reac-

Table I. R_M Values and NMR Spectral Characteristics of Key Compounds

compd	R_M[a]	^{1}H NMR, δ[b]
2	−0.05	1.04 (s, *t*-Bu), 2.34 (s, CH_2), 4.47 (s, Bz), 7.1–7.7 (m, Ar)
3	0.01	1.16 (s, *t*-Bu), 4.20 (s, CH), 4.46 (s, Bz), 7.1–7.7 (m, Ar)
4	−0.20	1.34 (s, *t*-Bu), 4.97 (br s, NH), 6.42 (s, Bz), 7.0–7.6 (m, Ar)
6	−0.13	1.32 (s, *t*-Bu), 4.76 (br s, NH), 5.03 (s, Bz), 6.9–7.4 (m, Ar)
8	−0.22	1.33 (s, *t*-Bu), 3.94 (s, Bz), 4.75 (br s, NH), 4.87 (s, CH_2-Fu), 7.2–7.4 (m, Ar)
10	−0.10	1.05 (s, *t*-Bu), 2.25 (s, CH_2), 5.19 (s, Bz)
12	−0.33	1.19 (s, *t*-Bu), 1.66 (m, CH_2-C), 2.26 (m, CH_2), 4.83 (br s, NH), 5.31 (s, CH_2-N)
14	−0.35	1.21 (s, *t*-Bu), 1.89 (s, Me), 2.13 and 2.67 (dd, CH_2-C), 2.79 and 2.82 (s, ring-CH_2), 4.8–4.9 (m, CH_2=), 4.98 (s, NH), 5.5–5.7 (m, CH=)
15	−0.40	2.81 (d, Me), 5.29 (d, NH), 6.40 (s, Bz), 7.0–7.5 (m, Ar)
26	−0.15	0.89 (s, *t*-Bu), 3.00 (d, CH_2), 5.02 (t, NH), 6.39 (s, Bz), 6.9–7.5 (m, Ar)
34	−0.29	2.97 (s, Me), 6.47 (s, Bz), 7.0–7.6 (m, Ar)
45	−0.21	1.66 (s, Me), 5.38 (s, NH), 6.29 (s, Bz), 7.0–7.5 (m, Ar)
49	0.05	1.03 (s, *t*-Bu), 2.26 (s, CH_2), 5.07 (s, Bz), 6.9–7.4 (m, Ar)
55	−0.02	1.43 (s, *t*-Bu), 3.48 (s, Bz), 6.9–7.4 (m, Ar)
61	−0.28	1.29 (s, *t*-Bu), 3.44 (s, Bz), 5.28 (br s, NH), 6.8–7.4 (m, Ar)
68	−0.07	0.94 (s, *t*-Bu), 3.11 (s, CH_2), 4.49 (s, Bz), 6.9–7.4 (m, Ar)

[a] Values obtained from reversed-phase TLC developed with acetone–water (4:1) are the mean of at least three determinations with a standard deviation of less than 0.04. [b] Spectra were measured in deuteriochloroform with tetramethylsilane as the internal standard. Abbreviations are as follows: s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet; br, broad; Ar, aromatic; Bz, benzylic methylene or methine; Fu, furyl; Ph, phenyl.

Pesticide Chemistry and Toxicology Laboratory, Department of Entomological Sciences, University of California, Berkeley, California 94720.

[1]Present address: Pesticide Research Laboratory, Takarazuka Research Center, Sumitomo Chemical Co., Ltd., 4-2-1 Takatsukasa, Takarazuka, Hyogo 665, Japan.

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