Residues of the Plant Growth Regulator Mefluidide [*N*-[2,4-Dimethyl-5-[[(trifluoromethyl)sulfonyl]amino]phenyl]acetamide] in the Milk and Tissues of Lactating Dairy Cows: A 28-Day Feeding Study

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Twelve lactating dairy cows received orally the plant growth regulator mefluidide at a treatment equivalent to 0, 6, 18, or 60 ppm of their diet/day during a 28-day feeding trial. The treatments had no apparent effect on the general health, appearance, feed consumption, milk production, or quality of the carcasses at slaughter in these dairy cows. Trace amounts of mefluidide were detected in the milk of all cows treated at 6 or 18 ppm, but with the exception of a single sample from a cow in the 18-ppm group, residues were all below the validated analytical limit of 0.005 ppm. Milk from cows treated with mefluidide at 60 ppm contained residues ranging from <0.005 to 0.015 ppm. There was no evidence of residue accumulation in milk, brain, muscle, liver, adipose tissue, or blood, and residues found in kidney samples (maximum 0.38 ppm) are probably associated with urinary elimination of mefluidide.

The plant growth regulator mefluidide [*N*-[2,4-dimethyl-5-[[(trifluoromethyl)sulfonyl]amino]phenyl]acetamide] (Figure 1) has shown potential for use in several agricultural and nonagricultural applications. Agricultural uses include the treatment of sugar cane to facilitate ripening and increase yields of recoverable sugar (Gates, 1975; Hagman et al., 1977; Zamora and Rosario, 1977), use as a herbicide to control Johnson grass and other weeds in soybeans and peanuts (Gates, 1975, 1976; Gruenhagen et al., 1975; Harrison et al., 1976), use on small grain crops, including wheat, to increase seed yields (Miles et al., 1977), utilization on certain pasture grasses to improve forage quality through suppression of seed head formation and increase in digestibility, sugars, and protein content (Glenn et al., 1980), and use as a sucker control agent in tobacco (Bandal, 1981).

Mefluidide has also proven useful and is registered for nonagricultural applications including (1) rights-of-way maintenance by highway departments, especially in difficult or hazardous-to-mow areas, (2) maintenance of grass areas in cemeteries reducing significantly the frequency of mowing and trimming around markers, shrubs, irrigation systems, and fences, (3) maintenance of grass areas along airport runways as an effective alternative to frequent mowing along hazardous runways and trimming around runway lights, hangers, and terminals, and (4) use as an alternative to frequent mowing of difficult-to-mow areas on golf courses (Bandal, 1981).

Although mefluidide has been shown to be low in toxicity to mammals and most other animal species (Bandal, 1981), it is imperative that its interactions with various components of the environment be definitely evaluated as part of the assessment of its suitability for use in agriculture. The proposed use patterns of mefluidide are such that livestock may be exposed to residues of the compound through consumption of sugar cane fractions (including molasses), soybeans, and wheat (including hay) and consumption of treated grass (through grazing or hay). The possibility thus exists that residues of mefluidide may enter the human food chain through contaminated meat or milk or products derived therefrom. The studies reported here were designed to define the potential for such residue transfer. Lactating dairy cattle were chosen as the experimental animals so that an evaluation could be made of potential residues that might appear in both the meat and milk of orally exposed livestock. Previous metabolic studies have shown that mefluidide is not appreciably metabolized in ruminants (Ivie, 1980), and thus analysis for only the parent compound provides an accurate measure of residues present.

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MATERIALS AND METHODS

Chemicals. For animal treatment, technical mefluidide (93% A.I.) was provided by 3M Co., St. Paul, MN, and its chemical identity and integrity were established by infrared, nuclear magnetic resonance, and mass spectroscopy and by gas–liquid chromatography. Upon receipt of the technical material, it was stored in the dark at 0 °C until use.

The hexane, acetonitrile, ethyl acetate, and chloroform were distilled in glass (Mallinkrodt Nanograde or equivalent). Anhydrous sodium sulfate was heated at 250 °C for 48 h and then stored in a glass-stoppered container until use. Diazomethane reagent was prepared from Diazald (Aldrich, Milwaukee, WI) by using procedure 1 given in the Aldrich bulletin for diazomethane preparation. Florisil, 100–200 mesh (Floridin Co.), was heated to 250 °C for 48 h and then adjusted to the desired activity by adding 10%, by weight, distilled water and mixing thoroughly. Deactivated Florisil was stored in a ground glass stoppered bottle until use.

Animals. Twelve lactating Holstein cows were selected from the Texas A&M University dairy herd. Other than apparent good health, the only selection criterion was that each of the animals must have produced an average of at least 25 lb of milk daily during the 4-week period prior to selection. Age, weight, breeding history, or stage of lactation were not considered in the selection process.

The twelve cows were randomly assigned to four treatment groups of three each and were individually identified and recognized by existing ear tag and/or brand numbers. Except during periods of feeding with dairy concentrate, treatment, milking, or examination, the animals were maintained as a group in a separate pen, isolated from all other animals at the University Dairy Center. The cows were provided hay (sorghum–sudan cross) and water ad libitum and just prior to each milking were individually stanchioned and provided dairy concentrate used in normal dairy operations at the Dairy Center. The amount of concentrate provided to each cow was adjusted periodically based on milk production, in accordance with standard dairy procedures, so that each animal received approxi-

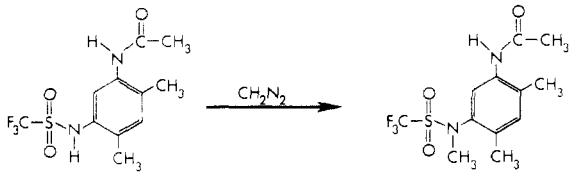

Figure 1. Structures of mefluidide and of its methylated analogue used for analysis of mefluidide residues in milk and tissues of dairy cattle.

mately 1 lb of concentrate/3 lb of milk produced.

Treatment. Animals in each of the four groups (three per group) were treated with mefluidide equivalent to the following concentrations in the total diet: 0, 6, 18, and 60 ppm of mefluidide. These levels were determined by considering the maximum anticipated mefluidide residues that livestock might consume if exposed to feed or forage contaminated with mefluidide as a result of its proposed agricultural uses. Based on known crop residue data previously obtained and evaluated (Bandal, 1981), it has been estimated that the maximum dietary exposure of livestock to mefluidide would approximate 6 ppm in the total diet (based on consumption of treated sugar cane and byproducts, soybeans and wheat and byproducts, forage or hay, or a mixture thereof). The studies reported here were designed to determine potential meat or milk residues resulting from exposure at 0×, 1×, 3×, and 10× the maximum anticipated daily intake. Thus, the mefluidide levels administered were 0, 6, 18, and 60 ppm based on total dietary intake. It was assumed for the purposes of this study that lactating dairy cattle consume 3% of their body weight per day as total feed intake of dry matter.

The animals were treated orally with the appropriate amounts of mefluidide administered in 1-oz gelatin capsules. The technical mefluidide was added to the capsules, along with a small amount of the dairy concentrate, the capsules were capped, and the material was mixed by shaking. Each cow was weighed on 3 successive days beginning not more than 7 days prior to initiation of the study, and this average weight was used as the basis for determining the amount of mefluidide to be administered throughout the study. During the 28-day treatment period, each animal received a total of 56 capsules. All of the capsules were prepared, capped, sealed, and individually identified prior to the initiation of the study and were held in the dark at 5 °C until just prior to treatment. Capsules for treatment of the control animals were prepared, numbered, and stored in the same manner as described above, except that they were filled only with dairy concentrate. The mefluidide or control-capsule treatments were administered with a balling gun twice daily, just prior to milking while the animals were stanchioned and receiving the allotted dairy ration. Initiation of the study began at an a.m. milking cycle, with the first capsules being administered just prior to a morning milking. Treatment then continued at each milking until each animal had been treated for 28 days.

Milking Schedule, Milk Collection, and Storage. The milking schedule was maintained as closely as possible to that which the animals were accustomed to as part of the University dairy herd. The morning milkings were at approximately 5–6 a.m. and the evening milkings at 3–4 p.m. After mefluidide treatment and consumption of the premilking ration, the cows were moved to the adjacent milking parlor, the udder and teats were washed thoroughly with warm water, and the animals were milked with vacuum-operated automatic milking machines. Sufficient milking units were not available to allow each animal to be milked by a separate unit, but so that possible contamination was avoided, the order of milking was in all cases such that the controls were milked first, followed by the animals in sequence with increasing mefluidide dose. The weights of milk produced by each animal at each milking were recorded by standard dairy procedure. After each milking cycle, the entire milking system was thoroughly cleansed with detergent washes and antiseptic treatment according to standard dairy procedure.

Although milk production was recorded at each milking, milk samples were collected for subsequent analysis only on days −1, 1, 3, 7, 10, 14, 17, 21, 24, and 28. The samples collected on day −1 (the day just prior to initiation of treatments) provided pretreatment milk from each of the animals. Day 1 is represented by a.m. and p.m. milk samples collected on the first day after initiation of the treatments, and thus the date of the first mefluidide treatments is defined as day 0.

For collection of samples for residue analysis, two 100–150-mL samples of the fresh whole milk were immediately drawn off into an 8-oz glass jar (precleaned with detergent wash and acetone rinse). The jars were then tightly sealed with polyseal caps and within 1 h transferred to a freezer and held until analysis. On the dates of milk collection, samples of both a.m. milk and p.m. milk were taken.

Slaughter of Animals, Collection, and Storage of Tissues. On the day after administration of the final capsule to each animal, the cows were milked in the morning on schedule but did not receive mefluidide treatment. They were then transported to slaughter facilities maintained by the University, stunned by captive-bolt, and killed by exsanguination. The following tissues were taken from each animal: blood (allowed to clot), muscle (loin), brain, kidney, liver, and fat (omental). Samples were placed in plastic bags and then in cartoons, fully labeled, and placed in a freezer until analyzed.

Residue Analysis. *Sampling and Extraction.* The samples of a.m. and p.m. milk were pooled to provide "whole day" samples for analysis. Pooling was accomplished by thawing the appropriate samples, mixing thoroughly, and combining appropriate ratios of the a.m. and p.m. samples, based on the quantity of milk produced during these periods. Pooled whole milk (100 mL) was placed in a 500-mL Nalgene centrifuge bottle, then 5 mL of 35% aqueous trichloroacetic acid solution was added, and the bottle was shaken for complete mixing. The acidified milk was extracted 3 times with 100-mL aliquots of ethyl acetate, and each time the layers were separated by centrifugation. Ethyl acetate extracts were combined in a 250-mL separatory funnel and then washed with 150-mL water containing 1 mL of 35% trichloroacetic acid and 5 g of NaCl. The extracted milk and the aqueous wash were discarded.

The ethyl acetate was removed by using a rotary evaporator with the water bath adjusted to 45 °C, and the nonvolatile material was transferred to a 250-mL separatory funnel containing 150 mL of 0.1 N NaOH. This alkaline solution was washed twice with 50-mL aliquots of chloroform and then acidified to pH 1 with 2 mL of concentrated HCl. The acidified solution was extracted 3 times with 50-mL aliquots of chloroform. The chloroform extract was dried with anhydrous Na_2SO_4, evaporated to about 15 mL under vacuum by using the rotary evaporator, and then transferred to the Florisil column for cleanup (vide infra).

Samples of nonfatty tissue (brain, muscle, liver, and kidney) were taken by cutting slices from various parts of the frozen tissue. Slices were made at random with no

preference or selection of the anatomical portion of the tissue samples. The only selection was to avoid particularly bloody or fatty areas or parts of the tissue which contained excessive proportions of connective tissue. The tissue sample (10 g) was placed in a 250-mL Nalgene centrifuge bottle and was extracted 3 times with 100-mL volumes of ethyl acetate by using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY). The homogenates were centrifuged between each homogenization step, and the ethyl acetate was transferred to a 500-mL round-bottom flask. The flask was then attached to a rotary evaporator, and the ethyl acetate was removed by vacuum evaporation at 45 °C. From this point, the procedure was as described for milk.

Samples of adipose tissue were taken by slicing random portions from the frozen tissue. The 10-g sample was further cut into small pieces and placed in a 150 × 28 mm (90 mL) test tube along with 1 g of Celite filter aid and 50 mL of ethyl acetate. This mixture was homogenized with a Polytron homogenizer and then centrifuged. The ethyl acetate was poured through a glass chromatography column containing approximately 15 g of anhydrous Na_2SO_4 and drained into a 250-mL round-bottom flask. The homogenization–centrifugation procedure was repeated twice to accomplish thorough extraction of the insoluble material that remained in the test tube. After evaporation of the ethyl acetate, the nonvolatile material was transferred to a 250-mL separatory funnel with 150 mL of hexane and was then extracted 3 successive times with 50-mL portions of acetonitrile. The combined acetonitrile phase was then washed with 200 mL of hexane, and all the hexane was discarded. The acetonitrile was drained into a 250-mL round-bottom flask and was removed by evaporation to dryness with a vacuum rotary evaporator (45 °C). The extract was then transferred to the Florisil cleanup column (vide infra).

Whole blood was thawed and mixed, and 10 g was placed in a 250-mL Nalgene centrifuge bottle, then 2 mL of 35% aqueous trichloroacetic acid solution were added, and the bottle was shaken for complete mixing. The acidified blood was extracted 3 times with 50-mL aliquots of ethyl acetate, and each time the layers were separated by centrifugation. The ethyl acetate extracts were combined in a 250-mL separatory funnel and then washed with 150 mL of water containing 1 mL of 35% trichloroacetic acid and 5 g of NaCl. The extracted blood and the aqueous wash were discarded. The ethyl acetate fraction was removed by using a vacuum evaporator (45 °C), and the residue was then transferred to the Florisil cleanup column (vide infra).

Elution Chromatography. Each column was prepared by adding in succession 6 g of 10% deactivated Florisil (100–200 mesh), 5 g of anhydrous Na_2SO_4, and a glass wool plug to a 150 × 28 mm glass chromatography column (Corning No. 8260). The Florisil was wetted with 25 mL of chloroform prior to addition of the extract. The extract of either milk, tissue, or blood was transferred to the column with 50 mL of chloroform and then 100 mL of ethyl acetate. Mefluidide was then eluted from the column into a 250-mL round-bottom flask with 100 mL of acetonitrile. The flask was attached to a rotary evaporator, and the acetonitrile was removed by vacuum evaporation at 45 °C.

Methylation of Mefluidide. Following evaporation of the acetonitrile eluate, the contents of the round-bottom flask were dissolved in 5 mL of diethyl ether, and then 5 drops of methanol and 0.5 mL of the diazomethane reagent were added. The solvent was evaporated by using "gentle" heat and a stream of clean, dry nitrogen. The methylated mefluidide (Figure 1) was then transferred to a 10-mL volumetric flask and diluted to the mark with ethyl acetate. All methylation procedures were conducted in a laboratory fume hood, and precautions were taken to avoid inhalation or dermal contact with diazomethane.

Gas Chromatographic Analysis. A Tracor MT-220 gas chromatograph (Tracor, Inc., Austin, TX) with a ^{63}Ni electron capture detector and detector linearizer was used for residue analysis. The signal from the detector was fed into an Autolab System IV computing integrator (Spectra-Physics, Mountain View, CA) as well as a Westronics 1-mV strip chart recorder (Westronics Inc., Fort Worth, TX).

The analytical column was a 0.6 m × 3 mm i.d. glass tube packed with 6% SP-2250 on Gas-Chrom Q (80–100 mesh) and was installed for direct on-column injection. Analyses were conducted with column temperature, injector temperature, and detector temperature set at 215, 250, and 300 °C, respectively. The carrier gas was 5% methane in argon (inlet pressure 40 psi) with a flow through the column of 75 cm^3/min and detector purge gas of 25 cm^3/min.

The mefluidide peak in tissue extracts was identified and quantitated by comparison with the response to an authentic mefluidide standard (3M Co., St. Paul, MN). Retention time and geometry of the peaks observed from extracts of spiked control samples or in test samples corresponded to the retention time and geometry of the peak produced after injection of methylated authentic mefluidide. Elution time for the methylated mefluidide was approximately 400 s, and an injection equivalent to 0.05 ng of mefluidide resulted in a peak of about 7% of full scale above background. Mefluidide residues were calculated from a linear regression curve by using a Hewlett-Packard 9815A programmable calculator on the basis of GLC peak area computed by the integrator.

Validation of the Analytical Method. The analytical method was validated down to 0.005 ppm for milk and 0.01 ppm for all other tissues. Known amounts of authentic mefluidide were added to control samples of milk or tissues, and the spiked samples were extracted and analyzed as described above.

RESULTS AND DISCUSSION

This study produced no apparent evidence that daily oral treatment with mefluidide as high as 60 ppm for 1 month produced any ill effects on the lactating dairy cows. There was no apparent effect on the general health, appearance, feed consumption, milk production, or quality of the carcasses at slaughter.

Previously reported data on the metabolism of mefluidide in a lactating cow and a sheep demonstrated that mefluidide was not metabolized appreciably in ruminants (Ivie, 1980), and, thus, analysis for only the parent compound mefluidide would provide an accurate measurement of residues in the milk and tissues of cattle exposed to mefluidide through forage consumption. Each of the residue analytical methods developed was validated for the recovery and analysis of mefluidide. This included validation for recovery of mefluidide from control samples spiked at 0.005 ppm in milk and as low as 0.01 ppm in brain, muscle, liver, kidney, adipose tissue, and blood. In addition, the method was validated at 0.02 ppm in kidney and at 0.02 and 0.05 ppm in liver, muscle, and brain. Average recovery of known amounts of mefluidide from control tissue ranged from about 77% to 144%, and in most instances, recovery was above 90% (Table I). Residues in milk (Table II) and in tissues and blood (Table III) are reported as ppm of mefluidide found and are not

Table I. Validation of the Analytical Method: Recovery of Mefluidide Added to Control Milk, Tissue, and Blood

tissue	mefluidide added, ppm	mefluidide recovered, %[a]	replicates
milk	0.005	93 ± 1.5	5
brain	0.010	77 ± 8.9	3
	0.020	143 ± 4.4	3
	0.050	100 ± 6.3	6
muscle	0.010	130 ± 5.8	3
	0.020	101 ± 16.1	4
	0.050	95 ± 2.8	6
liver	0.010	100 ± 11.6	3
	0.020	100 ± 2.9	3
	0.050	88 ± 11.3	3
kidney	0.010	103 ± 14.5	3
	0.020	122 ± 8.8	3
adipose	0.010	88 ± 4.8	4
blood	0.010	103 ± 6.3	4

[a] Mean ± standard error.

corrected for percent recovery. No evidence of mefluidide residues was observed in any of the pretreated samples or in any control samples.

The data on the mefluidide residues in milk indicate that at 6- and 18-ppm dose levels, orally administered mefluidide is not likely to be secreted into the milk at levels higher than 0.005 ppm. All milk samples at 6- and 18-ppm dose levels contained mefluidide residues at levels less than 0.005 ppm with only one exception. The 21-day milk sample from one cow at the 18-ppm dose level contained 0.006 ppm of mefluidide (Table II).

At the 60-ppm treatment level, mefluidide milk residues were low; the values ranged from less than 0.005 ppm to 0.015 ppm ($\bar{X}$ = 0.008 ppm), with no indication of accumulation. These data support the report by Ivie (1980) that orally administered [^{14}C]mefluidide [0.1–1.0 mg of mefluidide (kg of body weight)$^{-1}$ day^{-1}] was rapidly and completely excreted by a sheep and a cow, primarily in the urine, with no detectable radiocarbon being secreted into the milk.

Mefluidide residues were not detected in any of the brain and muscle samples at all doses except for one incidence with cow no. 670 at the 60-ppm dose level where mefluidide residues were detected at about 0.01 ppm (at the limit of analytical sensitivity) in the muscle tissue (Table III). No mefluidide residues were detected in the fat and blood samples at the 6- and 18-ppm dose levels except that 0.01 ppm of mefluidide occurred in the fat sample of one cow from the 18 ppm dose group. Oral administration of 60 ppm of mefluidide also resulted in very low levels of mefluidide residues in the fat and blood samples. These data clearly demonstrate that mefluidide residues are removed from the blood rapidly and that they

Table II. Mefluidide Residues[a] in Milk[b] of Lactating Dairy Cows Fed Mefluidide at Three Treatment Levels Twice Daily for 28 Days and Controls

	mefluidide residues, ppm, for treatment[c]											
	0 ppm			6 ppm			18 ppm			60 ppm		
day	cow no. 369	cow no. 411	cow no. 609	cow no. 355	cow no. 429	cow no. 680	cow no. 277	cow no. 520	cow no. 639	cow no. 227	cow no. 657	cow no. 670
−1	NDR[d]	NDR	NDR	NDR	NDR	NDR	NDR	NDR	NDR	NDR	NDR	NDR
1	NDR	NDR	NDR	<0.005[e]	<0.005	<0.005	<0.005	<0.005	<0.005	0.006	0.014	<0.005
3	NDR	NDR	NDR	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.008	0.013	0.005
7	NDR	NDR	NDR	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.009	0.013	<0.005
10										0.006	0.010	<0.005
14	NDR	NDR	NDR	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.006	0.014	0.005
17										0.007	0.009	<0.005
21	NDR	NDR	NDR	<0.005	<0.005	<0.005	0.006	<0.005	<0.005	0.005	0.015	0.005
24										0.006	0.013	0.005
28	NDR	NDR	NDR	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.007	0.015	0.007

[a] Reported as ppm (micrograms of mefluidide per milliliter of whole milk); not corrected for recovery; method validated at 0.005 ppm. [b] Composite sample made up by proportional pooling of a.m. and p.m. milk. [c] Cows were given mefluidide at the dose level indicated by oral capsule at each milking (twice daily). [d] No detectable residue. [e] <0.005: indicates the presence of detectable residue less than 0.005 ppm, the minimum residue level at which the analytical method was validated.

Table III. Mefluidide Residues in Tissues of Lactating Dairy Cows Fed Mefluidide Daily at Three Treatment Levels for 28 Days and Controls

cow no.	mefluidide treatment level, ppm[a]	mefluidide residues,[b] ppm, in indicated tissues					
		brain	muscle	liver	kidney	adipose	blood
369	0	NDR[c]	NDR	NDR	NDR	NDR	NDR
411	0	NDR	NDR	NDR	NDR	NDR	NDR
609	0	NDR	NDR	NDR	NDR	NDR	NDR
355	6	NDR	NDR	NDR	0.06	NDR	NDR
429	6	NDR	NDR	NDR	0.03	NDR	NDR
680	6	NDR	NDR	<0.01[d]	0.04	NDR	NDR
277	18	NDR	NDR	0.01	0.15	0.01	NDR
520	18	NDR	NDR	<0.01	0.10	NDR	NDR
639	18	NDR	NDR	NDR	0.33	NDR	NDR
227	60	NDR	NDR	0.03	0.38	0.03	0.04
657	60	NDR	NDR	<0.01	0.07	<0.01	0.02
670	60	NDR	0.01	0.02	0.34	<0.01	0.03

[a] Cows were given mefluidide at the level indicated by oral capsule at each milking (twice daily). [b] Reported as ppm (micrograms of mefluidide per gram of undried tissue); not corrected for recovery (Table I). [c] No detectable residue. [d] <0.01: indicates the presence of detectable residue less than 0.01 ppm, the minimum residue level at which the analytical method was validated.

do not show any appreciable potential for accumulation in the adipose tissue.

Analysis of the liver tissue from cows at all dose levels showed very low levels of detectable mefluidide residues; the maximum levels found were <0.01, <0.01, and <0.03 ppm in the 6, 18, and 60 ppm dose groups, respectively.

In the entire study, kidney was the only tissue that contained greater than 0.03 ppm of mefluidide residues. An average of 0.04, 0.19, and 0.26 ppm of mefluidide was found in kidneys of cows administered 6-, 18-, and 60-ppm doses of mefluidide, respectively. The presence of some mefluidide residues in the kidney might be predicted on the basis of findings by Ivie (1980) that an animal excreted the radiocarbon-labeled mefluidide rapidly and almost totally (>93%) via urine. In the radiotracer study, radiocarbon residues in the kidney of the cow corresponded to 0.005 ppm of mefluidide equivalents, with the sample being taken 5 days after the fifth and the final dose of [^{14}C]mefluidide. In the present 28-day study, however, the animals were sacrificed within 24 h of the final dosing with mefluidide. The results of these two studies suggest that the residues seen in the kidney will fall below GLC detectable levels (0.01 ppm) within a few days following withdrawal of mefluidide-treated feed.

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Flavones of *Scutellaria ovata*

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Seven flavones were isolated and identified from hot methanol extracts of roots, stems, and flowers of *Scutellaria ovata* (skullcap). Five of the compounds, chrysin, spigenin, luteolin, 6-methoxyluteolin, and dinatin, were previously known. Two new flavones, oroxylin 7-*O*-glucoside and ovatin (5,6-dimethoxyflavone 7-*O*-glucoside), were identified. Each compound was tested for its effect on radish root and stem growth. Chrysin reduced radish root growth by 20% at 100 ppm as compared with the water control.

The genus *Scutellaria* (skullcap) belongs to the Lamiaceae family. The chemistry of flavonoids from several species of *Scutellaria*, all of which are common to Europe, has been investigated. Bargellini (1919) found baicalein and baicalin (7-*O*-glucuronide of baicalein) in *Scutellaria baicalenis*. Bandyakova (1969) isolated the 7-β-D-glucuronoside, scutellarein, from *Scutellaria polyodon*. Denikeena et al. (1970) isolated and identified eight flavonoids from *Scutellaria przewalskii*. Litvinenko et al. (1971) identified baicalin and the glucosides of apigenin and luteolin, wogonin, apigenin, and luteolin from *Scutellaria scordifolia*. The phytotoxicity and structures of flavonoids of *Scutellaria* species indigenous to the United States that have not been investigated are the subject of this report.

MATERIALS AND METHODS

Extraction of *Scutellaria ovata*. Fresh plant material was collected and identified by the Botanical Institute of Mississippi State University. Roots, leaves, and flowers were separately steeped in hot water (50 °C) for 5 min to stop enzymatic activity. The water was decanted, and the plant material was finely ground in a blender with MeOH and heated at reflux for 2 h. The methanolic extract of flowers (600 g) yielded 5.0 g (0.8% FW) of solids. The methanolic extract of roots (1 kg) also yielded 5.0 g (0.5% FW) of solids.

Fractionation of Methanolic Flower Extract. The scheme for fractionation of the methanolic extract of *Scutellaria ovata* flowers is shown in Figure 1. The flower extract (5 g) was chromatographed on a Sephadex LH-20 column (l = 1 m, i.d. = 3 cm; solvent = MeOH) to give two fractions: A, 780 mg (15.5%), and B, 450 mg (9.0%). Fraction A was chromatographed on a silica gel column (l = 45 cm, i.d. = 3 cm; solvent = benzene–EtOAc, 8:2 v/v) to give A_1 (300 mg, 38.5%) and A_2 (200 mg, 25.6%). The collection of these two fractions was monitored by TLC plate (silica gel with the same solvent).

A_1 and A_2 were separately introduced into a DCC (Droplet countercurrent apparatus, Tokyo Rikakikai, Toyama-Cho, Kanda Chiyoda-ku, Tokyo, Japan). The upper phase of a solution of $CHCl_3$–MeOH–water (65:35:20 v/v/v) was the stationary phase, and the lower phase of

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