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# In Vivo Absorption, Distribution, Excretion, and Metabolism of a New Herbicide, Methiozolin, in Rats following Oral Administration

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**ABSTRACT:** Methiozolin [5-(2,6-difluorobenzyl)oxymethyl-5-methyl-3-(3-methylthiophen-2-yl)-1,2-isoxazoline] is a new turf herbicide that controls annual bluegrass in various cool- and warm-season turfgrasses. The present study is the first report elucidating absorption, tissue distribution, excretion, and metabolism of methiozolin in rats. The pharmacokinetic parameters in the blood were observed as follows: $t_{max}$ = 6 h, $C_{max}$ = 168.7 μg equiv/mL, $t_{1/2}$ = 49.4 h, $AUC_{120}$ = 9921.5 μg equiv·h/mL, and clearance = 39.2 mL/h/kg. Those parameters and the depletion curve for $^{14}C$ in the plasma were very similar to those in the blood. Total excretion through urine and feces was 24.3 and 68.9%, respectively, during 120 h after administration; however, there was no excretion through expired air. The radioactivity excreted through bile was 40.1% of that administered. Excreted radioactivity peaked between 24 and 48 h, showing 51.0% of total excretion within 48 h. The orally administered $^{14}C$ distributed across various tissues within 12 h after administration, showing 14.0% of the dosed, and was eliminated from all tissues without accumulation. Numerous minor metabolites (<4% of the dosed) in urine and fecal extract were detected within 72 h, and two of those were identified. The identified metabolites were Met-1 (glucuronic acid conjugate), 6-[5-(5-((2,6-difluorobenzyloxy)-methyl)-4,5-dihydro-5-methylisoxazol-3-yl)-4-methylthiophen-2-yloxy]-tetrahydro-3,4,5-trihydroxy-2*H*-pyran-2-carboxylic acid, and Met-2, [2-(5-((2,6-difluorobenzyloxy)methyl)-4,5-dihydro-5-methylisoxazol-3-yl)thiophen-3-yl]methanol. Conclusively, methiozolin was shown to be readily absorbed in the gastrointestinal tract, distributed throughout the tissues within 12 h, metabolized extensively, and eliminated through urine and feces mostly within 48 h, without tissue accumulation.

**KEYWORDS:** *methiozolin, pharmacokinetics, absorption, distribution, excretion, metabolism*

## INTRODUCTION

Methiozolin [5-(2,6-difluorobenzyl)oxymethyl-5-methyl-3-(3-methylthiophen-2-yl)-1,2-isoxazoline] was developed by Moghu Research Center and registered as a new turf herbicide in 2010 in Korea. This molecule was first invented as a rice herbicide candidate[1] but not commercialized. Hwang et al. reported that the molecule controls barnyardgrass (*Echinochloa* spp.), sedge weeds, and several other annual broad-leaved weeds in paddy condition.[2] In a later study, Koo and Hwang found that the herbicide had potent pre- and postemergence efficacies on annual bluegrass (*Poa annua*) and large crabgrass (*Digitaria sanguinalis*), with high safety to various warm- and cool-season turfgrasses including creeping bentgrass, Kentucky bluegrass, perennial ryegrass, zoysiagrass, and bermudagrass.[3] Lee et al. reported that methiozolin inhibited biosynthesis of both cellulose and hemicellulose fractions greatly.[4] However, the herbicidal symptom of methiozolin indicated that its mode of action was different from that of inhibitors of cellulose synthesis, microtubule disrupter, or inhibitors of very-long-chain fatty acids.[5] In a recent study, Grossman et al. suggested that methiozolin might inhibit tyrosine aminotransferase involved in the plastoquinone biosynthesis in duckweed (*Lemna paucicostata* L.).[6] Up to now, the mode of action of the herbicide might be directly or indirectly associated with cell wall biosynthesis and potentially plastoquinone biosynthesis in susceptible plants, but the primary site of herbicidal action is still unclear.

In various toxicological studies, methiozolin was shown to be practically nontoxic, having acute oral $LD_{50}$ of >2000 mg/kg body weight, NOAEL of 5000 mg/kg body weight in 90 days repeated dietary administration, and NOAEL of 1000 mg/kg body weight in prenatal development toxicity in rats.[7] However, it is unclear whether the low toxicity could be due to the inherent safety of the molecule, lack of absorption in gastrointestinal tracts, or metabolic detoxification. In this paper, basic information on the pharmacokinetic parameters, tissue distribution, excretion, and metabolism of methiozolin in rats following oral administration of $^{14}C$-labeled compound is reported.

## MATERIALS AND METHODS

**Test Animals.** Male Sprague−Dawley (SD) rats, 8−9 weeks old, were purchased from Koatech (Pyeongtaek, Korea). Animals were acclimatized for at least 1 week prior to administration. One day before administration, the animals were kept individually in a closed glass metabolism cage. Sterilized tap water and a laboratory rodent diet (Chunhajeil Feed Co., Daejeon, Korea) were given ad libitum to the animals throughout the experiment except for the one night before test substance administration.

**Test Condition and Metabolism Apparatus.** The rats were individually housed in the all-glass metabolism cages, which were designed to collect volatile products, carbon dioxide, urine, and feces separately. Carbon dioxide was collected with two traps of 1 N NaOH solution, whereas volatile compounds were collected in two traps

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containing ethylene glycol. The air was pulled through the closed system continuously by a vacuum pump at a flow rate of 400 mL/min. The urine and feces receivers were cooled in an ice vessel, and the whole system was kept in a controlled room at 23 ± 2 °C and 60 ± 10% relative humidity. The room was controlled under a 12 h day−night cycle (light from 8 a.m. to 8 p.m.).

**Test Materials.** The $^{14}$C-labeled methiozolin (specific activity = 6.59 MBq/mg, purity = 99.7%) was synthesized at Korea Radiochemicals Center (Suwon, Korea) and an unlabeled methiozolin (purity = 99.8%) was synthesized at Moghu Research Center Ltd. (Daejeon, Korea).[8] The chemical structure of methiozolin and $^{14}$C-labeled position are shown in Figure 1. Corn oil was purchased from

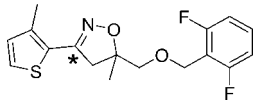


**Figure 1.** Chemical structure of methiozolin and $^{14}$C-labeled position.

Sigma Chemical Co. (St. Louis, MO, USA). The liquid scintillation cocktails (Ultima-Flo M, Insta-Gel Plus, Hionic-Fluor, and Permafluor E$^+$), carbon dioxide absorbent (Carbo-Sorb E), and tissue solubilizer (Soluene-350) were purchased from PerkinElmer (Waltham, MA, USA). All other solvents and reagents were commercial products of analytical grade.

**Measurement of Radioactivity.** Radioactivity was measured by a liquid scintillation counter (LSC; Tri-Carb 2900TR, PerkinElmer, USA). Radioactivity in gross amounts of less than twice the background was considered to be below the limit of determination accuracy. Aliquots (0.1 to 2 mL) of liquid samples were mixed with 7−12 mL of Insta-Gel Plus or Hionic-Fluor prior to LSC analysis. Portions of blood, plasma, and fecal residues were combusted using an automatic sample oxidizer (model 307, PerkinElmer, USA). The combustion products were absorbed into Carbo-Sorb E absorbent (5 mL) and mixed with Permafluor E$^+$ scintillator (10 mL). The efficiency of the oxidizer was determined using aliquots of Spec-Chec-$^{14}$C and was >95%. Measurements of radioactivity were corrected for oxidizer efficiency.

**Administration of Test Article.** The test article was prepared in a ratio of 125 mg of unlabeled methiozolin and 0.1 mg of $^{14}$C-methiozolin (0.66 MBq) per 1 mL of corn oil. It was suspended in corn oil, heated to 60 °C to obtain a completely dissolved solution, and cooled at room temperature before administration. Methiozolin in the cooled corn oil after heating was not chemically changed and not precipitated at room temperature during a day (data not shown). The rats were orally administered 1 mL of the test solution, which corresponded to a dose of 500 mg/kg body weight of a rat. This dose was a maximum dose level that did not show an acute clinical symptom and diarrhea in a preliminary study conducted in the same experimental condition. The average body weight of 18 rats tested in the following studies was 248.7 ± 6.4 g.

**Radioactivity in Blood and Plasma.** Blood samples (0.20−0.25 mL) were taken from the tail vein at 3, 6, 12, 24, 48, 72, 96, and 120 h after dosing to three rats. Immediately after sampling, the plasma was separated by centrifugation at 10000 rpm for 5 min. Aliquots of the blood or plasma (0.1 mL) were added to a combustion paper cup and air-dried. To the dried cup was added 0.1 mL of Combustaid (PerkinElmer, USA), which was combusted using the automatic sample oxidizer, and then radioactivity was measured by LSC.

**Urinary and Fecal Excretion.** Each of the three rats received single oral administration of 1 mL of the test solution. Immediately after administration, each rat was housed in a metabolism cage. Urine was collected at 6, 12, 24, 48, 72, 96, and 120 h after initiation. The total volume of each sample was measured. Aliquots (0.5−1 mL) of each sample were mixed with 7 mL of Insta-Gel Plus for radioactivity measurement with LSC. Feces were collected at 24, 48, 72, 96, and 120 h after initiation. The feces sample was extracted two times, using a homogenizer (Bio Homogenizer, ESGE, Switzerland), with a methanol/water (50:50, v/v) solution of at least 2-fold the volume of the sample and then ultrasonicated for 15 min at ambient temperature. The homogenate was centrifuged at 5000 rpm for 15 min, and duplicate aliquots of 1 mL of each supernatant were mixed with 7 mL of Insta-Gel Plus for measurement of radioactivity. The debris of the extracted feces was dried at 40 °C and then homogenized. Triplicate portions (0.2 g) of the feces powder were combusted using the automatic sample oxidizer, and then radioactivity was measured by LSC. Expired air was passed through two traps containing 250 mL of 1 N NaOH solution for trapping carbon dioxide and two traps of 250 mL of ethylene glycol for trapping volatile compounds and collected at 12, 24, 48, and 72 h after administration. No radioactivity was detected in either trap solution until 72 h and, thus, was not collected after then. Following collection of the feces and urine, each metabolism cage was washed with acetone/water (50:50, v/v) solutions at 120 h after administration, and then duplicate aliquots (4 mL) of the washing solutions were mixed with 12 mL of Insta-Gel Plus for radioactivity measurement.

For quantification and identification of metabolites, an aliquot (100−200 μL) of the pooled urine and fecal extracts collected at each sampling time was analyzed using a radio-HPLC (200 series, PerkinElmer, USA) equipped with a UV−vis detector and a flow scintillation analyzer (Radiomatic 610TR, PerkinElmer, USA). The detection wavelength was 254 nm, and a reverse phase $C_{18}$ column (Cosmosil, 250 × 4.6 mm i.d., 5 μm, Nacalai Tesque, Japan) was used. The elution solvents were methanol and water (0.1% formic acid). A linear gradient (from 30 to 90% methanol for 50 min) using a flow rate of 1.0 mL/min was adopted to separate the peaks of methiozolin and potential metabolites produced. The flow rate of scintillation cocktail (Ultima-Flo M) was 3 mL/min.

**Bile Excretion.** Three rats were individually bile-duct cannulated and administered 1 mL of the test article solution. Each rat was housed in a metabolism cage. Bile was collected from each animal into a separately ice-cooled receiver at 6, 12, 24, 48, 72, 96, and 120 h after initiation. The total volume of each sample was measured. Aliquots (0.5−1 mL) of each sample were mixed with 7 mL of Insta-Gel Plus for radioactivity measurement and 100−200 μL was analyzed by the radio-HPLC.

**Tissue Distribution.** Each of nine rats was orally administered 1 mL of the test solution and then anesthetized by diethyl ether inhalation at 12, 48, and 120 h after administration. At each time, the following tissues were obtained from three rats for radioactivity determination: adrenal glands, bladder, bone marrow, brain, epididymis, gastrointestinal tract (GIT), heart, kidneys, liver, lungs, pancreas, pituitary gland, spleen, testes, and thyroid. The separated tissue samples were weighed, chopped, and solubilized in 2−10 mL of Soluene-350 at 60 °C. After complete solubilization, the samples were cooled at room temperature and 2−10 mL of methanol was added, and then aliquots (0.5−2 mL) were mixed with 7−12 mL of Hionic-Fluor for radioactivity measurement.

**Isolation of Metabolites from Feces.** In the present study, isolation and identification of the metabolites were conducted in feces, but not in urine because there were no major metabolites potentially subjected to isolation in the radio-HPLC chromatogram. The feces collected at 24, 48, and 72 h was separately extracted as described above, and the fecal extracts were combined and then concentrated in vacuo. The concentrated extracts was injected to the HPLC equipped with a column, 250 × 10 mm i.d., 15 μm, Cosmosil 5$C_{18}$-MS-II (Nacalai Tesque, Japan) and eluted by methanol and water (0.1% formic acid) in a linear gradient (from 30 to 90% methanol for 40 min). The detection wavelength was 254 nm, and the flow rate was 4.0 mL/min. The eluted solution corresponding to a metabolite peak was collected and concentrated in vacuo. To obtain a single metabolite from the separated eluents, further purification was conducted by thin layer chromatography (TLC) using precoated silica gel 60 $F_{254}$ chromatoplates (20 × 20 cm, 0.5 mm layer thickness, Merck, Darmstadt, Germany). The solvent systems were dichloromethane/methanol/acetic acid (6:1:0.6, v/v/v) and dichloromethane/methanol/acetic acid (9:1:0.9, v/v/v, developed in triplicate). Each metabolite separated by TLC was extracted with tetrahydrofuran, and the solvent was evaporated in vacuo until complete dryness. Finally, two purified metabolites were identified by NMR spectroscopic and MS spectrometric analysis.

**Spectroscopic and Spectrometric Analysis.** The chemical structures of purified metabolites were identified by nuclear magnetic

resonance (NMR) spectrometer (Bruker, Germany) operating at 500 MHz for $^{1}H$ and at 125 MHz for $^{13}C$ with $CDCl_3$ (99.8%, Merck) and $CD_3OD$ (99.9%, Cambridge Isotope Laboratories, Andover, MA, USA) as solvent, respectively, at room temperature. ESI negative spectra were recorded on 15Tesla Fourier transform ion cyclotron resonance mass spectrometer (15T FT-ICR MS, Bruker Daltonics, Germany) for Met-1 and EI-MS spectra on JMS-700 MS (JEOL, Japan) for Met-2, respectively.

**Data Analysis.** Mean and standard deviation calculations were performed using Microsoft Office Excel 2007 (Microsoft Corp., Redmond, WA, USA), and the total $^{14}C$ blood concentration−time data were analyzed using WinNonlin Pro software, version 3.3 (Pharsight Corp., St. Louis, MO, USA).

## RESULTS AND DISCUSSION

**Pharmacokinetic Parameters.** The $^{14}C$ concentration in the blood and plasma during 120 h following a single oral administration of the test article at 500 mg/kg body weight is shown in Figure 2. The estimated pharmacokinetic parameters

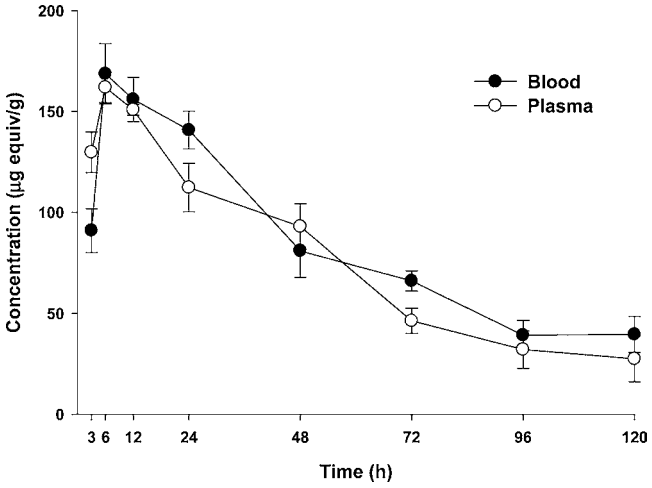


**Figure 2.** Concentration of $^{14}C$ in the blood and plasma of rats following a single oral administration of methiozolin at 500 mg/kg body weight. Each data point represents the mean ± SD.

such as the time showing a maximum concentration ($t_{max}$, h), maximum concentration ($C_{max}$, μg equiv of methiozolin/mL), half-life ($t_{1/2}$, h), $AUC_{120}$ (area under the concentration−time curve from 0 to 120 h), and clearance (Cl, mL/h/kg) were calculated (Table 1).

**Table 1. Estimated Main Pharmacokinetic Parameters of $^{14}C$ in Blood and Plasma after Oral Administration of $^{14}C$-Methiozolin at a Dose of 500 mg/kg Body Weight**

| | parameters[a] | | | | |
|---|---|---|---|---|---|
| | $C_{max}$ (μg/mL) | $t_{max}$ (h) | $t_{1/2}$ (h) | $AUC_{120}$ (μg·h/mL) | Cl (mL/h/kg) |
| blood | 168.7 | 6 | 49.4 | 9921.5 | 39.2 |
| plasma | 162.0 | 6 | 41.7 | 8943.2 | 47.2 |

[a] $C_{max}$, maximum concentration; $t_{max}$, time of $C_{max}$; $t_{1/2}$, elimination half-life; $AUC_{120}$, area under the concentration−time curve; Cl, clearance.

$t_{max}$ and $t_{1/2}$ were 6 and 49.4 h for the blood, respectively, indicating that administered $^{14}C$ was rapidly absorbed from the GIT and eliminated from the body with time. The estimated pharmacokinetic parameters between for blood and plasma were similar. In addition, the depletion curve for $^{14}C$

**Table 2. Concentration, Proportion, and Tissue to Blood Ratio following Oral Administration of $^{14}C$-Methiozolin at a Dose of 500 mg/kg Body Weight to Rats**

| | μg equiv of methiozolin/g wet tissue (mg/kg) | | | | | | proportion (% of administered dose) | | | | | | tissue to blood ratio | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 12 h | | 48 h | | 120 h | | 12 h | | 48 h | | 120 h | | 12 h | | 48 h | | 120 h | |
| tissue | mean | SD | mean | SD | mean | SD | mean | SD | mean | SD | mean | SD | mean | SD | mean | SD | mean | SD |
| blood | 156.08 | 25.14 | 81.02 | 18.02 | 39.64 | 8.96 | | | | | | | | | | | | |
| adrenal glands | 283.50 | 45.32 | 33.90 | 2.23 | 3.48 | 1.76 | 0.013 | 0.001 | 0.002 | <0.001 | <0.001 | <0.001 | 1.82 | 0.29 | 0.42 | 0.03 | 0.09 | 0.04 |
| bladder | 177.39 | 37.91 | 88.15 | 10.15 | 7.00 | 2.05 | 0.021 | 0.007 | 0.008 | 0.002 | <0.001 | <0.001 | 1.14 | 0.24 | 1.09 | 0.13 | 0.18 | 0.05 |
| bone marrow | 101.83 | 18.78 | 4.69 | 1.91 | 1.27 | 0.30 | 0.009 | 0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.65 | 0.12 | 0.06 | 0.02 | 0.03 | 0.01 |
| brain | 7.01 | 1.50 | 2.45 | 0.69 | 0.71 | 0.14 | 0.012 | 0.003 | 0.004 | 0.004 | 0.001 | <0.001 | 0.04 | 0.01 | 0.03 | 0.01 | 0.02 | <0.01 |
| epididymis | 16.29 | 0.53 | 15.83 | 1.17 | 1.79 | 0.41 | 0.010 | <0.001 | 0.008 | 0.005 | 0.001 | <0.001 | 0.10 | <0.01 | 0.20 | 0.01 | 0.05 | 0.01 |
| GIT[a] | 1413.30 | 201.55 | 218.77 | 32.34 | 11.46 | 3.93 | 11.684 | 1.291 | 1.915 | 0.054 | 0.096 | 0.028 | 9.05 | 1.29 | 2.70 | 0.40 | 0.29 | 0.10 |
| heart | 44.06 | 6.73 | 9.03 | 2.58 | 3.08 | 0.70 | 0.037 | 0.015 | 0.008 | 0.005 | 0.003 | 0.001 | 0.28 | 0.04 | 0.11 | 0.03 | 0.08 | 0.02 |
| kidneys | 55.58 | 15.04 | 108.93 | 0.91 | 12.08 | 3.90 | 0.096 | 0.039 | 0.202 | 0.010 | 0.020 | 0.012 | 0.36 | 0.10 | 1.34 | 0.01 | 0.30 | 0.10 |
| liver | 180.21 | 39.81 | 165.10 | 31.96 | 21.99 | 6.11 | 1.469 | 0.383 | 1.638 | 0.833 | 0.168 | 0.038 | 1.15 | 0.26 | 2.04 | 0.39 | 0.55 | 0.15 |
| lungs | 374.70 | 40.92 | 32.34 | 0.13 | 5.95 | 1.63 | 0.525 | 0.381 | 0.048 | <0.001 | 0.009 | 0.002 | 2.40 | 0.26 | 0.40 | 0.00 | 0.15 | 0.04 |
| pancreas | 57.23 | 10.73 | 24.07 | 5.05 | 4.23 | 1.14 | 0.058 | 0.041 | 0.050 | 0.053 | 0.009 | 0.003 | 0.37 | 0.07 | 0.30 | 0.06 | 0.11 | 0.03 |
| pituitary gland | 307.61 | 25.28 | 15.23 | 3.27 | 4.17 | 1.55 | 0.003 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 1.97 | 0.16 | 0.19 | 0.04 | 0.11 | 0.04 |
| spleen | 10.81 | 3.16 | 11.23 | 1.68 | 5.39 | 1.07 | 0.006 | 0.002 | 0.008 | 0.004 | 0.003 | 0.001 | 0.12 | 0.03 | 0.22 | 0.08 | 0.07 | 0.01 |
| testes | 5.78 | 1.88 | 5.60 | 1.13 | 1.29 | 0.34 | 0.018 | 0.005 | 0.019 | 0.009 | 0.004 | 0.001 | 0.07 | 0.02 | 0.14 | 0.02 | 0.14 | 0.03 |
| thyroid | 12.50 | 2.10 | 9.47 | 1.80 | 1.89 | 0.30 | 0.006 | 0.003 | 0.004 | 0.002 | 0.001 | <0.001 | 0.04 | 0.01 | 0.07 | 0.01 | 0.03 | 0.01 |

[a] Gastrointestinal tract (not including contents).


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Table 3. Mean Daily and Cumulative Percent of $^{14}C$ in Urine and Feces after Oral Administration of Methiozolin at a Dose of 500 mg/kg Body Weight to Rats[a]

| | % of administered radioactivity | | | | | |
|---|---|---|---|---|---|---|
| | urine | | feces | | total | |
| time (h) | daily | cumulative | daily | cumulative | daily | cumulative |
| 0–6 | 3.54 ± 1.96 | 3.54 ± 1.96 | NA | NA | 3.54 ± 1.96 | 3.54 ± 1.96 |
| 6–12 | 2.51 ± 1.38 | 6.05 ± 0.77 | NA | NA | 2.51 ± 1.38 | 6.05 ± 0.77 |
| 12–24 | 5.02 ± 1.44 | 11.07 ± 2.00 | 26.95 ± 1.50 | 26.95 ± 1.50 | 31.96 ± 1.48 | 38.01 ± 2.25 |
| 24–48 | 8.46 ± 2.41 | 19.52 ± 4.31 | 31.12 ± 4.70 | 58.07 ± 3.28 | 39.58 ± 5.94 | 77.59 ± 5.45 |
| 48–72 | 2.79 ± 0.85 | 22.32 ± 4.07 | 8.18 ± 4.48 | 66.24 ± 2.42 | 10.97 ± 5.15 | 88.56 ± 1.99 |
| 72–96 | 0.85 ± 0.21 | 23.17 ± 3.88 | 1.72 ± 0.18 | 67.96 ± 2.48 | 2.57 ± 0.31 | 91.13 ± 1.69 |
| 96–120 | 0.46 ± 0.20 | 23.63 ± 3.69 | 0.92 ± 1.03 | 68.88 ± 1.50 | 1.38 ± 0.85 | 92.51 ± 2.42 |
| cage wash | 0.70 ± 0.44 | 24.33 ± 4.01 | NA | NA | 0.70 ± 0.44 | 93.21 ± 2.66 |
| expired air | | | | | ND | ND |
| total | | 24.33 ± 4.01 | | 68.88 ± 1.50 | | 93.21 ± 2.66 |

[a]Values were expressed as the mean ± SD; NA, not applicable; ND, not detected.

concentration in the plasma was very similar to that in the blood. This result suggests that methiozolin or its metabolites were not accumulated in blood cells.

**$^{14}C$ Distribution in Tissues.** Radioactivity distributed rapidly to all tissues within 12 h after administration and declined with time thereafter (Table 2). The only exception was kidney. Concentration of $^{14}C$ in kidney (108.93 μg equiv/g) was highest at 48 h after dosing. At 12 h after administration, the $^{14}C$ concentration in blood was 156.08 μg equiv/mL, and the concentrations in most tissues were similar to or lower than the blood concentration. Certain tissues showed higher concentrations than the blood; these include GIT (1413.30 μg equiv/g), lungs (374.70 μg equiv/g), pituitary gland (307.61 μg equiv/g), and adrenal glands (283.50 μg equiv/g). The lowest concentration was measured in testes (5.78 μg equiv/g). Relatively high proportions of radioactivity were measured in GIT (11.684%), liver (1.469%), lungs (0.525%), and kidneys (0.096%) at 12 h. At 120 h after administration, the concentrations of $^{14}C$ in most tissues were several times lower than those at 12 h, suggesting methiozolin or its metabolites were easily eliminated. The lower radioactivity in the brain (0.71 μg equiv/g) at 120 h indicated that methiozolin or its metabolites have low permeability on the blood–brain barrier.[9]

In the present study, the radioactivity was distributed across various tissues, indicating that methiozolin or its metabolites were permeated to all tissues. The tissue to blood ratio (tissue concentration/blood concentration) was much higher especially in GIT, lungs, pituitary gland, and adrenal gland at 12 h; however, the values in all tissues declined to 0.02–0.55 at 120 h after administration. These results suggest that the measured $^{14}C$ might have low lipophilicity and be eliminated easily without accumulation in the tissues.[10]

**Excretion in Feces and Urine.** The overall recovery of total radioactivity from urine and feces was 93.2% of the administered radioactivity (Table 3). Within the first 48 h, 77.6% of the administered radioactivity was excreted in urine (19.5%) and feces (58.1%), and these amounts accounted for >83% of the total recovered radioactivity from urine, feces, and cage wash during the entire period. After 120 h, 24.3 and 68.9% of the administered radioactivity were excreted through urine and feces, respectively. When the vapor pressure of the chemical is higher than $1 \times 10^{-5}$ mmHg (20 °C), trapping of volatile products is recommended,[11] and ethylene glycol,[12] cold ethanol,[13] dry ice–acetone,[14] Tenax GC, and silica gel[15] have been used to trap the volatiles in general. Although the vapor pressure of methiozolin is $1.9 \times 10^{-13}$ mmHg at 25 °C,[7] traps of ethylene glycol for volatile compounds were used because some volatile products may be generated as metabolites during the study. However, no volatile product was detected during the major absorption and excretion period (0–72 h) after dosing, suggesting that volatile metabolites might not be produced from the rats administered methiozolin.

The results suggest that methiozolin is rapidly absorbed and excreted in the first 48 h followed by slow residual excretion. Cumulated recovery was >93% of the administered, proving the experiment was conducted in a valid condition, and the result would represent the true excretion kinetics. The radioactivity recovered in the urine and feces suggested methiozolin is excreted through the fecal and urinary system, but not through the respiratory system. Excreted radioactivity was almost 3-fold greater in the feces than in urine, suggesting fecal excretion might be the more important route of elimination of methiozolin in rats. In previous excretion studies with several agrochemicals, compounds such as pyribenzoxim[12] and pyridalyl,[16] having relatively low water solubility, were excreted through feces mostly, whereas more highly water-soluble compounds such as clothianidin[17] and furametpyr[18] were excreted more through urine. Water solubilities of pyribenzoxim, pyridalyl, clothianidin, and furametpyr were 3.0 mg/L, 0.15 μg/L, 340 mg/L, and 225 mg/L,[19] respectively. Methiozolin has relatively low water solubility (1.6 mg/L),[20] and the result of major excretion through the feces is comparable to the previous studies with pyribenzoxim and pyridalyl.

**Biliary Excretion.** The cumulative bile excretion during the 120 h was 40.1% of the administered dose (Figure 3). The majority of the bile excretion (94.3% of cumulative bile excretion for 120 h or 37.8% of that administered) was measured within the first 48 h after dosing. The excretion rate in feces and urine from the bile-cannulated rats was not determined in this study; however, the absorption rate (64.4%) and the direct fecal excretion rate (28.8%) could be estimated by adding the urinary and the biliary excretion and by deducting the biliary excretion from the fecal excretion, respectively.


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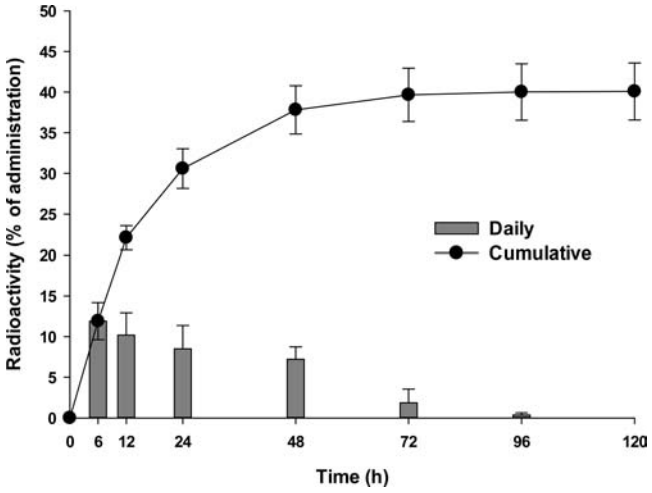


**Figure 3.** Daily and cumulative amounts of radioactivity excreted from bile of rats after oral administration of methiozolin at 500 mg/kg body weight. Each data point represents the mean ± SD.

Biliary excretion is well-known to be a major pathway for the elimination of amphipathic, hydrophobic, and high molecular weight xenobiotics[21] and can be linked to a high clearance.[22] In previous studies, molecular weight was a dominant factor influencing biliary excretion,[23−25] and foreign compounds having high molecular weight (>325 ± 50) were excreted in bile.[26,27] On the basis of this study, extensive biliary excretion of methiozolin suggests that the produced metabolites might have a high molecular weight.

**Metabolites in Urine, Feces, and Bile.** Typical radio-HPLC chromatograms of $^{14}$C-methiozolin, feces extract, urine, and bile are shown in Figure 4. The amount of metabolite was calculated using the radioactivity according to the metabolite peak integrated by the radio-HPLC, and the value was corrected by multiplying the dilution rate (total sample volume/injection volume to the radio-HPLC). Methiozolin was detected only in the feces extract and was calculated at 2.2% of that administered. In the feces extract and urine, numerous minor metabolites were present, but the amount of each metabolite was measured below 4% of that dosed. In bile, at least three metabolites were found at proportions of 3.9−10.9% of that administered during 72 h. These results implied that the absorbed methiozolin was rapidly biotransformated into numerous minor metabolites and excreted through urine and feces in rats.

In this study, two metabolites from the fecal extracts were identified by NMR and MS spectrometry and were designated Met-1 and Met-2, respectively. Met-1 and Met-2 were detected during 72 h after administration and accounted for 3.08 and 1.79% of that dosed, respectively. The highest amounts of Met-1 and Met-2 were measured at 1.63 and 0.92% of that administered during 0−24 h, respectively. These results indicated that both metabolites were produced and excreted rapidly within the first 24 h and that the production and excretion lasted until 72 h after administration.

**Identification of Isolated Metabolites.** The NMR data and chemical structures of the identified metabolites, Met-1 and Met-2, are shown in Table 4 and Figure 5, respectively.

*Met-1.* In $^{1}$H NMR, three proton signals at $\delta$ 6.97 (2H) and 7.38 (1H) and two protons signals at $\delta$ 3.03 and 3.35 were indicated the presence of the difluorobenzene ring and isoxazole ring, respectively. Four protons signals at $\delta$ 3.52 (2H) and 4.69

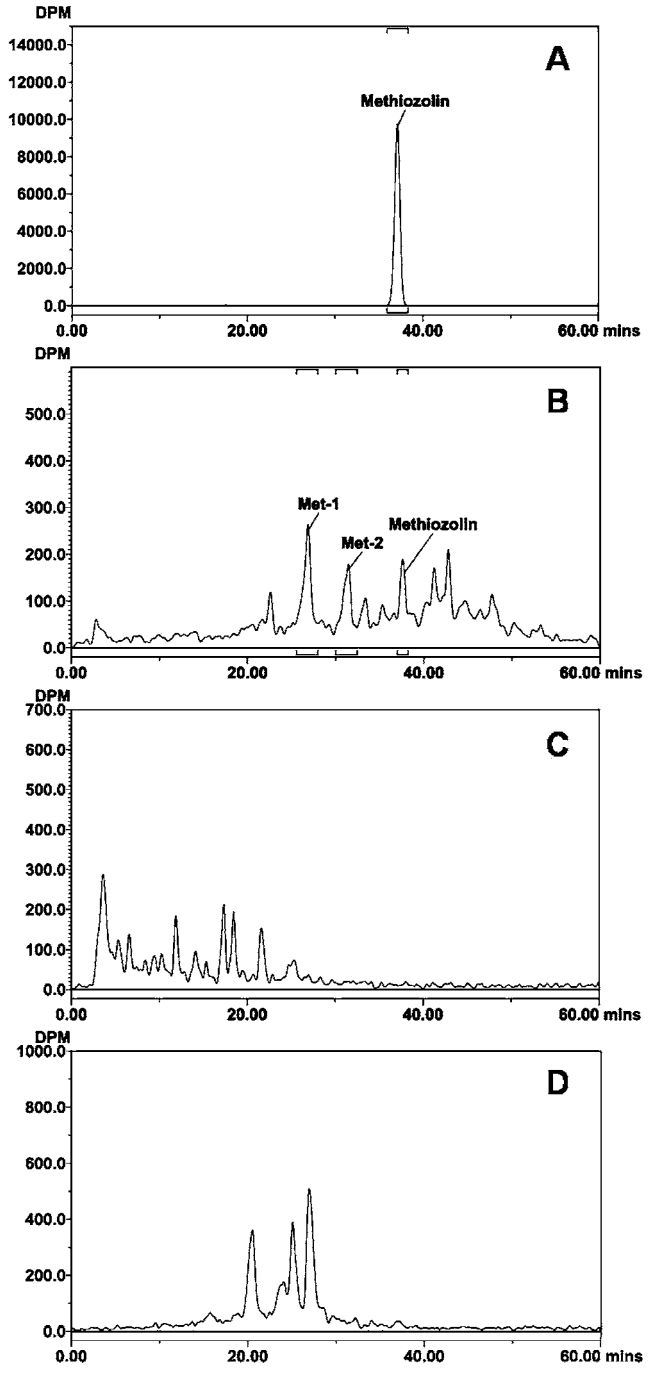


**Figure 4.** Representative radio-HPLC chromatograms for $^{14}$C-methiozolin stock solution (A), fecal extracts (B), urine (C), and bile (D) sampled within 24 h after oral administration to rats.

(2H) were assigned to the ether bond. Three protons signals at $\delta$ 1.38 were assigned to the methyl moiety of the isoxazole ring. Three protons signals at $\delta$ 2.27 (3H) were assigned to the methyl moiety of the thiophene ring. One proton signal at $\delta$ 6.91 of C2 in thiophene ring disappeared compared to methiozolin, and five protons signals ($\delta$ 4.83, 3.80, 3.35, 3.46, and 3.55) for glucuronic acid were observed. The signals in $^{13}$C NMR for Met-1 were assigned as follows: $\delta$ 132.1 (C2), 132.2 (C3), 138.7 (C4), 16.8 (C5), 116.0 (C6), 162.9 (C7), 46.2 (C10), 74.5 (C11), 23.2 (C12), 87.5 (C13), 75.6 (C15), 164.0 (C17), 112.5 (C18), 132.1 (C19), 112.5 (C20), and 154.6 (C21). Six carbons signals for glucuronic acid were observed as


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**Table 4. $^{1}H$ and $^{13}C$ NMR Data for Methiozolin, Met-1, and Met-2**

| $^{1}H$ NMR Data | | | | | | |
|---|---|---|---|---|---|---|
| | methiozolin | | Met-1 | | Met-2 | |
| proton | $\delta$ ($CDCl_3$) | $J$ (Hz) | $\delta$ ($CDCl_3$) | $J$ (Hz) | $\delta$ ($CD_3OD$) | $J$ (Hz) |
| 2 | 6.91 (m) | | *b* | | 7.30 (m) | |
| 2-1 | *b* | | 4.83 (d) | 7.2 | *b* | |
| 2-3 | *b* | | 3.80 (d) | 9.9 | *b* | |
| 2-8 | *b* | | 3.35 (s) | | *b* | |
| 2-9 | *b* | | 3.46 (m) | | *b* | |
| 2-11 | *b* | | 3.55 (m) | | *b* | |
| 3 | 7.28 (m) | | 6.40 (s) | | 7.08 (m) | |
| 5 | 2.46 (3H, s) | | 2.27 (3H, s) | | 4.70 (2H, s) | |
| 5[a] | *b* | | *b* | | 4.16 (br) | |
| 10 | 3.02 (d) | 16.45 | 3.03 (dd) | 3.6 | 2.98 (d) | 16.64 |
| | 3.45 (d) | 16.50 | 3.35 (m) | | 3.52 (d) | 16.62 |
| 12 | 1.47 (3H, s) | | 1.38 (s) | | 1.45 (3H, s) | |
| 13 | 3.55 (2H, dd) | 9.85, 9.90 | 3.52 (2H, m) | | 3.56 (2H, m) | |
| 15 | 4.72 (2H, s) | | 4.69 (2H, 2H) | | 4.70 (2H, s) | |
| 18[c] | 6.91 (m) | | 6.97 (2H, m) | | 6.89 (m) | |
| 19 | 7.28 (m) | | 7.38 (m) | | 7.30 (m) | |
| 20[c] | 6.91 (m) | | 6.97 (2H, m) | | 6.89 (m) | |
| $^{13}C$ NMR Data | | | | | | |
| | methiozolin | | Met-1 | | Met-2 | |
| carbon | $\delta$ ($CDCl_3$) | $J$ (Hz) | $\delta$ ($CDCl_3$) | $J$ (Hz) | $\delta$ ($CD_3OD$) | $J$ (Hz) |
| 2 | 126.5 | | 132.2 | | 128.6 | |
| 2-1 | *d* | | 105.9 | | *d* | |
| 2-3 | *d* | | 77.6 | | *d* | |
| 2-5 | *d* | | 176.6 | | *d* | |
| 2-8 | *d* | | 73.3 | | *d* | |
| 2-9 | *d* | | 75.6 | | *d* | |
| 2-11 | *d* | | 61.8 | | *d* | |
| 3 | 125.8 | | 132.1 | | 126.4 | |
| 4 | 138.7 | | 138.7 | | 143.1 | |
| 5 | 16.4 | | 16.8 | | 59.1 | |
| 6 | 125.8 | | 116.0 | | 128.6 | |
| 7 | 152.5 | | 162.9 | | 152.2 | |
| 10 | 45.5 | | 46.2 | | 44.7 | |
| 11 | 74.3 | | 74.5 | | 73.9 | |
| 12 | 23.3 | | 23.2 | | 23.0 | |
| 13 | 85.9 | | 87.5 | | 86.7 | |
| 15 | 60.9 | | 75.6 | | 60.3 | |
| 16 | 113.3 | | 112.5 | | 113.3 | |
| 17[e] | 160.9 | | 164.0 | | 161.2 | |
| 18 | 111.4 | | 112.5 | | 111.2 | |
| 19 | 131.9 | | 132.1 | | 130.4 | |
| 20 | 111.4 | | 112.5 | | 111.2 | |
| 21[e] | 163.3 | | 154.6 | | 162.6 | |

[a]OH. [b]No proton. [c]H–F coupling. [d]No carbon. [e]C–F coupling.

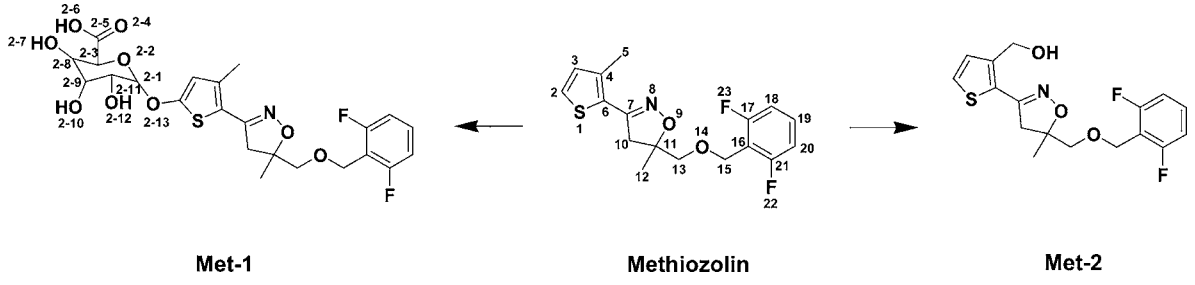


**Figure 5.** Chemical structures of methiozolin and identified metabolites in rats.


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follows: δ 105.9 (C2-1), 77.6 (C2-3), 176.6 (C2-5), 73.3 (C2-8), 75.6 (C2-9), and 61.8 (C2-11). The ESI negative spectrum showed a molecular ion peak at *m/z* 528.12 ($[M - H]^-$). From the MS/MS analysis, an increase of *m/z* 352.08 produced by glucuronyl moiety cleavage from Met-1 was observed (data not shown). On the basis of these results, Met-1 was identified as a glucuronic acid conjugate, 6-[5-(5-((2,6-difluorobenzyloxy)methyl)-4,5-dihydro-5-methylisoxazol-3-yl)-4-methylthiophen-2-yloxy]tetrahydro-3,4,5-trihydroxy-2*H*-pyran-2-carboxylic acid.

*Met-2*. In $^1$H NMR, three protons signals at δ 6.89 (2H) and 7.30 (1H) and two protons signals at δ 2.98 and 3.52 (2H, *J* = 16.64, 16.62 Hz) indicated the presence of a difluorobenzene ring and an isoxazole ring, respectively. Four protons signals at δ 3.56 (2H) and 4.70 (2H) were assigned to the ether bond. Three protons signals at δ 1.45 were assigned to the methyl moiety of the isoxazole ring. Two protons signals at δ 7.30 (1H) and 7.08 (1H) was assigned to the thiophene ring. However, two protons signals at δ 2.46 (3H) of methiozolin were shifted to δ 4.70 (2H) and a signal at δ 4.16 (OH) was observed. The results indicated that the methyl moiety (C5) of the thiophene ring was hydroxylated. In the $^{13}$C NMR spectrum, the signals were assigned as follows: δ 128.6 (C2), 126.4 (C3), 143.1 (C4), 59.1 (C5), 128.6 (C6), 152.2 (C7), 44.7 (C10), 73.9 (C11), 23.0 (C12), 86.7 (C13), 60.3 (C15), 113.3 (C16), 161.2 (C17), 111.2 (C18), 130.4 (C19), 111.2 (C20), and 162.6 (C21). One carbon signal of the C5 position was changed compared to that of methiozolin. EI-MS gave a molecular ion peat at *m/z* 353 ($M^+$) and their fragment peaks (*m/z* 196, 178, 127, and 111). From the results, the chemical structure of Met-2 was identified as [2-(5-((2,6-difluorobenzyloxy)methyl)-4,5-dihydro-5-methylisoxazol-3-yl)-thiophen-3-yl]methanol. On the basis of the identification of the two metabolites, the biotransformation reactions are proposed to be hydroxylation of the methyl moiety and glucuronic acid conjugation in the thiophene ring of methiozolin.

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### Notes

The authors declare no competing financial interest.

## REFERENCES


(1) Ryu, E. K.; Kim, H. R.; Jeon, D. J.; Song, J. W.; Kim, K. M.; Lee, J. N. ; Kim, H. C.; Hong, K. S. Preparation of herbicidal 5-benzyloxymethyl-1,2-isoxazoline derivatives of weed control in rice. Patent WO 200209185, 2002.

(2) Hwang, I. T.; Kim, H. R.; Jeon, D. J.; Hong, K. S.; Song, J. H.; Cho, K. Y. 5-(2,6-Difluorobenzyl)oxymethyl-5-methyl-3-(3-methylthiophen-2-yl)-1,2-isoxazoline as a useful rice herbicide. *J. Agric. Food Chem.* **2005**, *53*, 8639−8643.

(3) Koo, S. J.; Hwang, K. H. Use of 5-benzyloxymethyl-1,2-isoxazoline derivatives as herbicide. U.S. Patent 2008/0318784 A1, 2008.

(4) Lee, J. N.; Koo, S. J.; Hwang, K. H.; Hwang, I. T.; Jeon, D. J.; Kim, H. R. Mode of action of a new isoxazoline compound. *Proc. 21st APWSS Conf.* **2007**, 597−601 (Colombo, Sri Lanka).

(5) Koo, S. J.; Hwang, K. H.; Jeon, M. S. Methiozolin − a new turf herbicide. *WSSA Meet. Abstr.* **2008**, 43.

(6) Grossmann, K.; Hutzler, J.; Tresch, S.; Christiansen, N.; Looser, R.; Ehrhardt, T. On the mode of action of the herbicides cinmethylin and 5-benzyloxymethyl-1,2-isoxazolines: putative inhibitors of plant tyrosine aminotransferase. *Pest Manag. Sci.* **2011**, *68*, 482−492.

(7) Koo, S. J.; Hwang, K. H.; Jeon, M. S; Kim, S. H.; Lim, J.; Lee, D. G.; Chung, K. H.; Ko, Y. K.; Ryu, J. W.; Koo, D. W.; Woo, J. C. Development of the new turf herbicide methiozolin. *Kor. J. Weed Sci.* **2010**, *30*, 323−329.

(8) Nam, J. H.; Hwang, K. H.; Koo, S. J.; Kim, C. H.; Cho, C. W.; Ko, Y. K. Synthesis and herbicidal activities of enantiopure methiozolins. *Bull. Korean Chem. Soc.* **2012**, *33*, 297−300.

(9) Hughes, W. W. Distribution and storage of toxicants. In *Essentials of Environmental Toxicology*; Simon, C., Twigg, S. M., Dugger, E., Eds.; Taylor and Francis: Washington, DC, 1996; pp 59−69.

(10) Magnuson, B. A.; Appleton, J.; Ames, G. B. Pharmacokinetics and distribution of [$^{35}$S]methylsulfonylmethane following oral administration to rats. *J. Agric. Food Chem.* **2007**, *55*, 1033−1038.

(11) Martin, B. K. Pharmacokinetics. In *Handbook of Clinical Drug Research*; Glenny, H., Nelmes, P., Eds.; Blackwell: London, UK, 1986; pp 59−93.

(12) Liu, K. H.; Moon, J. K.; Sung, H. J.; Kang, S. J.; Koo, S. J.; Lee, H. S.; Kim, J. H. *In vivo* pharmacokinetics of pyribenzoxim in rats. *Pest Manag. Sci.* **2011**, *57*, 1155−1160.

(13) Mathews, J. M.; Parker, M. K.; Matthews, H. B. Metabolism and disposition of diethylene glycol in rat and dog. *Drug Metab. Dispos.* **1991**, *19*, 1066−1070.

(14) Marco, G. J.; Simoneaux, B. J.; Williams, S. C.; Cassidy, J. E.; Bissig, R.; Muecke, W. Radiotracer approaches to rodent dermal studies. In *Dermal Exposure Related to Pesticide Use*; Honeycutt, R. C., Zweig, G., Ragsdale, N. N., Eds.; American Chemical Society: Washington, DC, 1985; pp 43−61.

(15) Tanaka, A.; Watanabe, M. Distribution and metabolism of 1-propyl-1-nitrosourea in rats. *Cancer Res.* **1982**, *42*, 3837−3842.

(16) Nagahori, H.; Tomigahara, Y.; Isobe, N.; Kaneko, H. Metabolism of pyridalyl in rats: excretion, distribution, and biotransformation of dichloropropenyl-labeled pyridalyl. *J. Agric. Food Chem.* **2009**, *57*, 10845−10851.

(17) Yokota, T.; Mikata, K.; Nagasaki, H.; Ohta, K. Absorption, tissue distribution, excretion, and metabolism of clothianidin in rats. *J. Agric. Food Chem.* **2003**, *51*, 7066−7072.

(18) Nagahori, H.; Yoshino, H.; Tomigahara, Y.; Isobe, N.; Kaneko, H.; Nakatsuka, I. Metabolism of furametpyr. 2. $^{14}$C excretion, $^{14}$C concentrations in tissues, and amounts of metabolites in rats. *J. Agric. Food Chem.* **2000**, *48*, 5760−5767.

(19) *The Pesticide Manual*, 13th ed.; BCPC: Alton, UK, 2003.

(20) Korea Institute of Toxicology. Water solubility of methiozolin (EG11001), 2011.

(21) Vore, M. Phase III elimination: another two-edge sword. *Environ. Health Perspect.* **1994**, *102*, 422−423.

(22) Arimori, K.; Kuroki, N.; Hidaka, M.; Iwakiri, T.; Yamsaki, K.; Okumura, M.; Ono, H.; Takamura, N.; Kikuchi, M.; Nakano, M. Effect of P-glycoprotein modulator, cyclosporine A, on the gastrointestinal excretion of irinotecan and its metabolite SN-38 in rats. *Pharm. Res.* **2003**, *20*, 910−917.

(23) Wright, W. E.; Line, V. D. Biliary excretioin of cephalosporins in rats: influence of molecular weight. *Antimicrob. Agents Chemother.* **1980**, *17*, 842−846.

(24) Proost, J. H.; Roggeveld, J.; Wierda, J. M.; Meijer, D. K. Relationship between chemical structure and physicochemical properties of series of bulky organic cations and their hepatic uptake and biliary excretion rates. *J. Pharmacol. Exp. Ther.* **1997**, *282*, 715−726.

(25) Han, Y. H.; Kato, Y.; Haramura, M.; Ohta, M.; Matsuoka, H.; Sugiyama, Y. Physicochemical parameters responsible for the affinity of methotrexate analogs for rat canalicular multispecific organic anion transporter (cMOAT/MRT2). *Pharm. Res.* **2001**, *18*, 579−586.

(26) Hakk, H.; Larsen, G.; Bergman, A.; Orn, U. Metabolism, excretion and distribution of the flame retardant tetrabromobisphenol-A in

conventional and bile-duct cannulated rats. *Xenobiotica* **2000**, *30*, 881−890.
(27) Mathews, J. M.; Decosta, K.; Thomas, B. F. Lauramide diethanolamine absorption, metabolism, and disposition in rats and mice after oral, intravenous, and dermal administration. *Drug Metab. Dipos.* **1996**, *24*, 702−710.