JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2003,** *51,* 5977−5984 **5977**

Toxicokinetics, Recovery, and Metabolism of Metamitron in Goat

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Toxicokinetic behavior and metabolism studies of metamitron and its effect on the cytochrome P_{450} content of liver microsomal pellet were carried out in black Bengal goats after a single oral administration at 278 mg kg^{-1} and consecutive oral administration of 30 mg kg^{-1} for 7 days. Metamitron was detected in the blood sample at 0.08 h (12.0 ± 0.87 μg mL^{-1}), maximum at 4 h (84.3 ± 8.60 μg mL^{-1}) and minimum (14.6 ± 1.67μg mL^{-1}) at 36 h blood sample after a single oral administration. The absorption rate constant was 0.69 ± 0.09 h^{-1}. The Vd_{area} (2.00 ± 0.08 L kg^{-1}) and $t_{1/2}\beta$ (8.98 ± 0.70 h) values suggested wide distribution and long persistence of the compound in the body. The values of T ∼ B (0.80 ± 0.04), F_c (0.55 ± 0.01), Cl_B (0.15 ± 0.00 L kg^{-1} h^{-1}), and K_{21} (0.41 ± 0.03 h^{-1}) suggested that metamitron retained in the blood compared to that in the tissue. Maximum concentration of metamitron residue was found in the adrenal gland followed by bile on day 4 of single oral administration. The higher Cl_R compared to Cl_H value indicated the excretion of the major portion (34−40%) through urine compared to feces (20−26%). Maximum concentrations of metamitron and its metabolite, deaminometamitron, were excreted through urine and feces at 48 and 24 h samples, respectively. The recovery of metamitron including its metabolite in terms of parent compound varied from 69.3 to 80.1%, of which contribution of metabolite in terms of parent compound varied from 53.1 to 63.0%. Repeated oral administration of metamitron at 30 mg kg^{-1} for 7 days caused induction of the cytochrome P_{450} content of liver microsomal pellet of goat, suggesting oxidative deamination of metamitron.

KEYWORDS: Toxicokinetics; recovery; metamitron; metabolite; cytochrome P_{450}; goat

INTRODUCTION

Metamitron [4-amino-3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one] is widely used as a pre- and postemergence herbicide for the control of grasses and broad-leaved weeds in sugar and red beets, fodder beet, and certain strawberry varieties. The herbicidal property of metamitron was first reported in 1975 by Schmidt et al. (*1*). The photodecomposition of metamitron in soil has been demonstrated, and in water, photolysis produced deamination to deaminometamitron (*2*). However, information with respect to disposition kinetics and metabolism of metamitron especially in animal systems is not available in the literature. The objective of the study was to analyze the disposition kinetics behavior, distribution pattern, and metabolism of metamitron including the identification of its metabolite in an animal system. Black Bengal goat is a small ruminant used for human consumption and accordingly has been considered for this study. The data generated thereof will reveal the kinetics behavior, distribution, metabolism in goat, and possible indication of the safe use of the compound in human as well as animal society.

EXPERIMENTAL PROCEDURES

Chemicals. Metamitron (technical grade, purity = 98%) and its metabolite, 3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one (purity = 98%) were supplied by M/S Gharda Chemicals Ltd., Mumbai, India. These compounds were further purified (98.8%) and authenticated by TLC followed by HPLC and spectroscopic (UV, IR, MS, and NMR) analyses. All of the solvents and chemicals used in this study were of analytical grade and purchased from E. Merck and Sigma Chemical Co.

Animal Treatment. Clinically healthy adult black Bengal male and female (nulliparous) goats of ∼1.5−2 years age, weighing between 12 and 18 kg, were selected. The goats were kept in stainless steel metabolic cages. The room in which the animals were housed was provided with artificial fluorescent lighting and controlled temperature (22 ± 3 °C). Animals were fed with standard feed (*3*) (two parts wheat

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10.1021/jf034541u CCC: $25.00
Published on Web 08/22/2003

husk, one part ground nut cake, one part crushed gram, one part crushed maize, and two parts green) and water ad libitum. Each animal was fasted overnight before treatment. For ascertaining the minimum oral toxic dose level, four different dose levels of metamitron suspended in carboxymethylcellulose (CMC: 1% w/v in water) were administered by gavage to four groups of goats separately; each group comprised one male and one female. For metabolism study of metamitron, 14 male and 14 female goats were used. Of these, two males and two females were kept as control and received CMC in water only. The remaining goats were randomly divided into four groups, each made up of three male and three female animals, which were administered by gavage with metamitron suspension in CMC water. A group of treated goats was sacrificed on each of days 4, 5, 6, and 7. For ascertaining the maximum nontoxic oral dose level, three different dose levels of metamitron suspended in 1% CMC in water were administered by gavage once daily for 7 consecutive days to three groups of goats, each group comprising one male and one female goat. For microsomal study of metamitron, four male and four female goats were considered and divided randomly into two equal groups, each having two male and two female goats.

Determination of a Single Minimum Oral Toxic Dose. The objective was to find a dose level of metamitron that would produce minimum toxicity and no mortality within an observation period of 7 days after oral administration. Metamitron at four dose levels (222, 278, 370, and 445 mg kg^{-1}) suspended in CMC water (1% w/v) was administered by gavage once only to each of the animals of four groups, each consisting of one male and one female, respectively. They were then kept under observation. Metamitron at 222 mg kg^{-1} did not exhibit any toxic symptoms, whereas a dose level of metamitron at 278 mg kg^{-1} produced toxic symptoms in goats without mortality. The toxic symptoms were depression, drowsiness, grinding of teeth, and dyspnoea within 2 h; in addition, bradycradia, decreased rate of respiration, and formation of bloat were observed within 3 h; and there was a decrease in the frequency of urination and defecation at 12 h after administration.

All of the above-mentioned toxic symptoms disappeared 24 h after administration. Goats treated with metamitron at 370 and 445 mg kg^{-1} showed the above symptoms with greater intensity. Accordingly, the single minimum oral toxic dose level of metamitron has been fixed at 278 mg kg^{-1} in the present study.

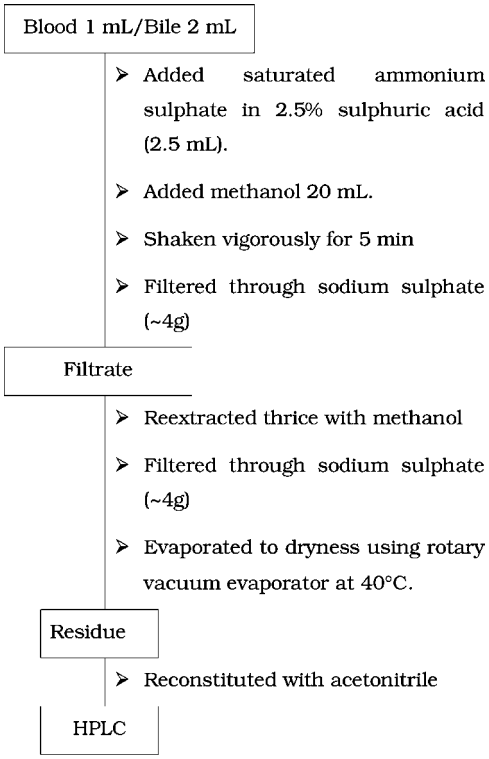
Determination of Maximum Nontoxic Oral Dose. The objective was to find a dose level of metamitron that on single oral administration for 7 consecutive days would not produce any toxic symptoms. Metamitron at three dose levels (20, 30, and 40 mg kg^{-1}) suspended in CMC (1% w/v) water was administered by gavage once daily for 7 consecutive days to corresponding three groups of goats, respectively. Dose levels of metamitron at 20 and 30 mg kg^{-1} did not produce any toxic symptoms, whereas the 40 mg kg^{-1} dose produced toxic symptoms such as depression, drowsiness, irritation to skin, apathy to motion, and anorexia within 10 min of the last dosing. Accordingly, metamitron at 30 mg kg^{-1} has been fixed as the maximum nontoxic oral dose.

Kinetics. For the kinetic study, blood samples were collected from the jugular vein of each experimental goat into heparinated tubes at different time intervals (0.08, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36, and 48 h) after metamitron administration in duplicate sets of test tubes separately. The concentration of metamitron was estimated by HPLC.

The values of kinetic parameters such as K_a (absorption rate constant), $t_{1/2}K_a$ (absorption half-life), β (elimination rate constant), $t_{1/2}\beta$ (elimination half-life), Vd_{area} (apparent volume of distribution), T ~ B (tissue blood ratio), AUC (total area under the blood herbicide concentration versus time curve), K_{el} (first-order rate constant of herbicide elimination from the central compartment), Cl_B (body clearance), Cl_R (renal clearance), Cl_H (hepatic clearance), K_{12} (rate constant for transfer of herbicide from the central to the peripheral compartments), K_{21} (rate constant for transfer of herbicide from peripheral to central compartment), and F_c (fraction of herbicide in the central compartment) were determined from semilogarithmic plots of mean blood concentration time profile data in goats using standard formulas (*4*, *5*).

Collection of Urine and Feces. Urine and feces were collected from individual goats at 24, 48, 72, 96, 120, 144, and 168 h after oral administration. The excretions were measured or weighed and stored at −20 °C prior to extraction.

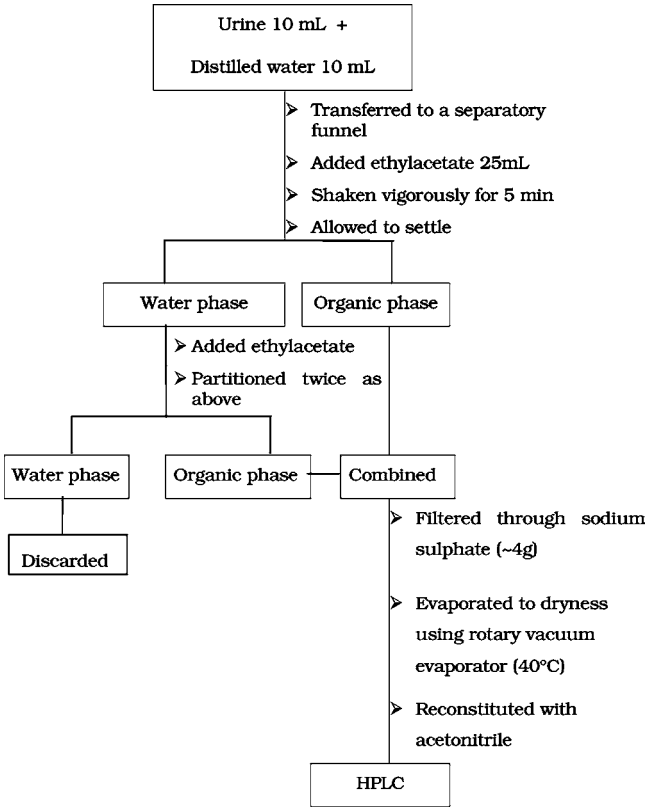
Scheme 1. Extraction and Analysis of Caprine Blood and Bile



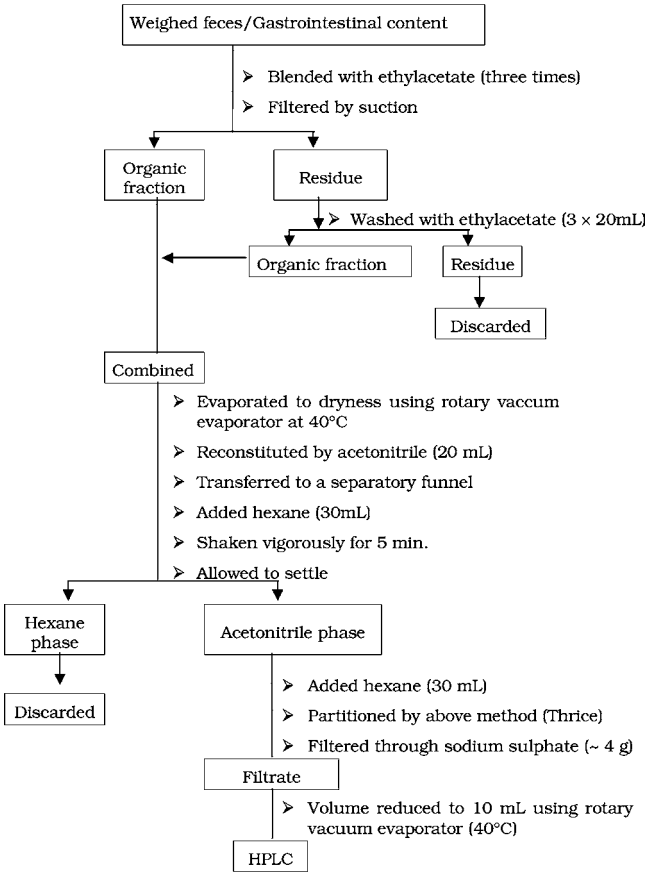
Collection of Tissues, Bile, and Gastrointestinal Contents. The animals of the first, second, third, and fourth groups were killed on days 4, 5, 6, and 7 after dosing, respectively. Samples of liver, kidney, lung, brain, heart, spleen, adrenal gland, omental fat, ovary, uterus, testis, rumen, reticulum, omasum, abomasum, intestine, bile, contents of rumen and intestine, and thigh muscle, bone, and skin were taken, weighed, minced, packed, and stored at −20 °C prior to extraction. A sample (2 g) of each of the above-mentioned tissues except bile (2 mL) and urine (10 mL) was extracted with respective solvent, and metamitron and its metabolite were quantified and thereafter multiplied with respective factors to get the total recovery. Because it is difficult to collect and weigh the whole muscle, skin, and bone, a representative sample (2 g for muscle and skin and 30 g for bone) of each was extracted with respective solvent (**Scheme 4**), quantified, and multiplied with factors as described by Davis et al. (*6*) to get the total recovery.

Microsomal Study. Metamitron at 30 mg kg^{-1} suspended in CMC water (1% w/v) was administered by gavage once daily for 7 consecutive days to each goat of group 2, whereas only CMC water was administered by gavage in the aforesaid procedure to each goat of group 1 (control). Goats of groups 1 and 2 were sacrificed on day 8, and pieces of liver (caudate lobe) were taken, trimmed of fascia, minced, and washed (three times) with ice-cold 1.15% KCl within 10 min; subsequent steps were carried out at 4 °C. The minced tissue was blotted, weighed, mixed with 4 volumes of buffer [tris(hydrochloride) (10 mM, pH 7.4) containing KCl (0.1 M), ethylenediaminetetraacetic acid (1.0 mM), and butylated hydroxytoluene (20 μM)], and homogenized in a mechanically driven Teflon−glass homogenizer (Remi RQ 127 A). The homogenate was centrifuged at $10^4 g$ in an automatic high-speed refrigerated centrifuge (SCR 20 B, rotor RPR 20-2) for 30 min, and the supernatant was centrifuged at 105000*g* for 1 h in a micro-ultracentrifuge (Hitachi CS-120Gx, rotor 100 AT4) to yield a microsomal pellet. The microsomal pellet was suspended in buffer [potassium pyrophosphate (0.1 M, pH 7.4) containing ethylenediaminetetraacetic acid (1.0 mM) and butylated hydroxytoluene (20 μM)], homogenized with four passes of a mechanically driven Teflon−glass homogenizer, and again recentrifuged at 105000*g* for 1 h. The supernatant fraction was decanted, the microsomal pellet was resuspended in a volume of buffer [tris(hydrochloride) (10 mM, pH 7.4), ethylenediaminetetraacetic

Scheme 2. Extraction and Analysis of Caprine Urine

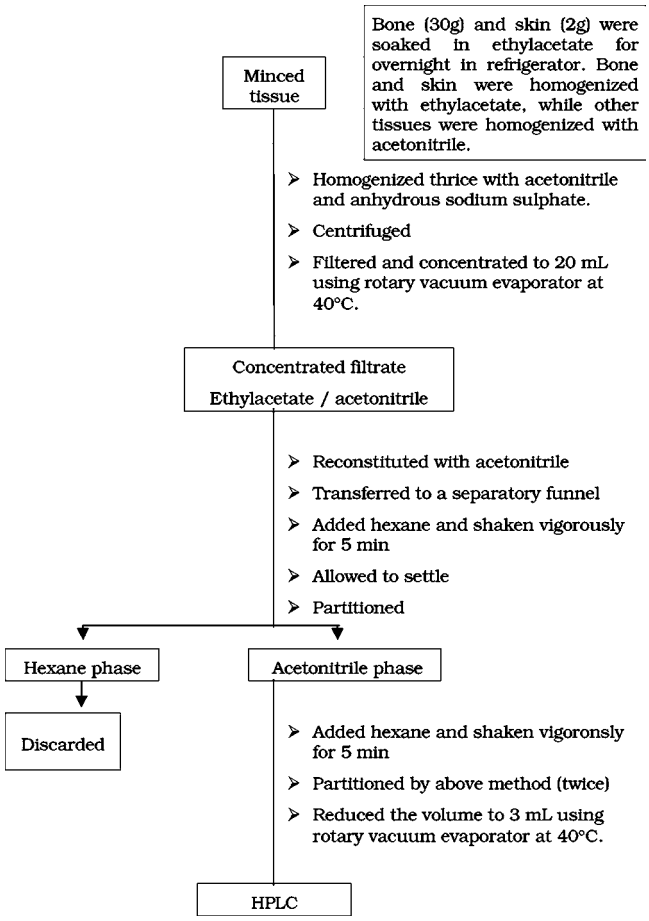


Scheme 3. Extraction and Analysis of Caprine Feces and Gastrointestinal Contents



acid (1.0 mM), and glycerol (20% v/v)], and cytochrome P_{450} was immediately measured. Protein was measured according to the method described by Lowry et al. (*7*).

Scheme 4. Extraction and Analysis of Caprine Tissue, Bone, and Skin



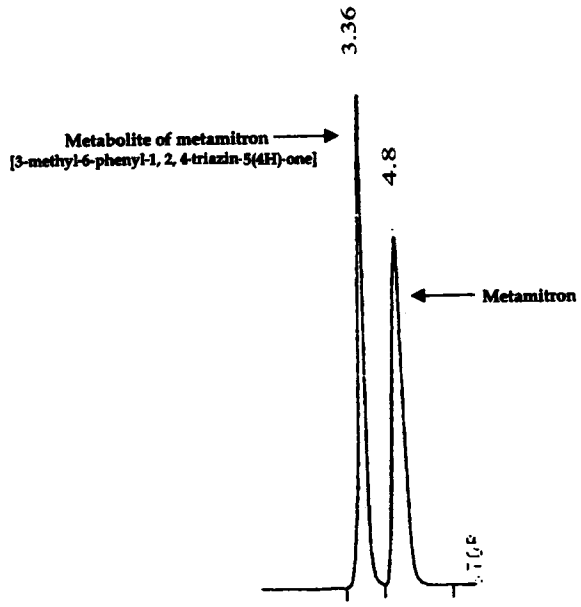
Extraction and Cleanup. *Blood and Bile.* To each sample of blood (1 mL) or bile (2 mL) was added saturated ammonium sulfate in 2.5% sulfuric acid (2.5 mL), and the mixture was shaken. The whole content was extracted with methanol (20 mL) and filtered through sodium sulfate (~4 g). The test tubes were washed three times with methanol (3 × 20 mL) and each time passed through sodium sulfate. The total filtrate was evaporated to dryness using a rotary vacuum evaporator at 40 °C. The final volume was made up with acetonitrile (HPLC grade) for HPLC analysis (**Scheme 1**).

Urine. To each urine sample (10 mL) was added and mixed distilled water (10 mL), and the mixture was transferred to a dry clean separatory funnel (125 mL). Ethyl acetate (25 mL) was added, shaken vigorously for 5 min, and allowed to settle for 2−3 min until the ethyl acetate phase was distinctly separated. The lower urine phase was again partitioned with ethyl acetate. Thereafter the procedure was as described in **Scheme 2**.

Feces. The method of extraction from feces and intestinal and ruminal contents is presented in **Scheme 3**.

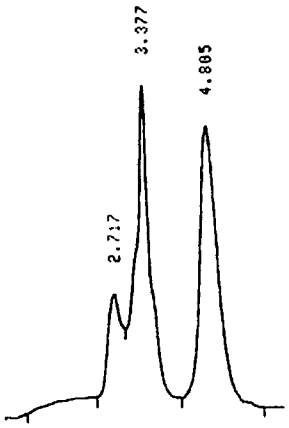
Tissues. The method of extraction and cleanup of tissue sample is presented in **Scheme 4**. A small amount of minced tissue (2 g) except bone and skin was homogenized with acetonitrile (20 mL), whereas crushed bone (30 g) and minced skin (2 g) were soaked in ethyl acetate (50 mL) and kept overnight in a refrigerator and then homogenized with ethyl acetate (20 mL).

Quantification. A Hewlett-Packard (model 1050) liquid chromatograph coupled with a variable-wavelength UV−vis detector attached to a 3392A integrator was used for the analysis of metamitron and its metabolite with the following operational parameters: column, Spherisorb, 125 × 4 mm, ODS2 5 μm, made of stainless steel; mobile phase, acetonitrile/water (1:1); the mixture was subjected to membrane filtration and degassed by ultrasonification; λ value, 306 nm; flow rate, 0.3 mL min^{-1}; injection using a 25 μL loop with a Hamilton syringe.

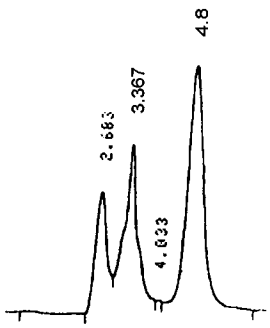


1.(a)

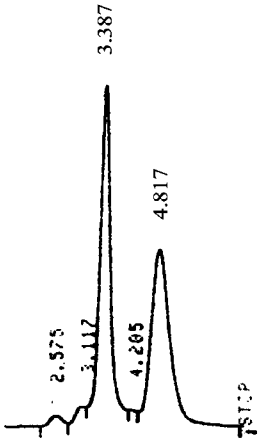
1.(b) i



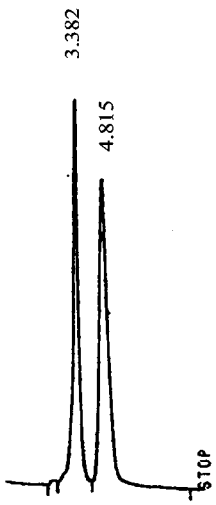
1.(b) ii



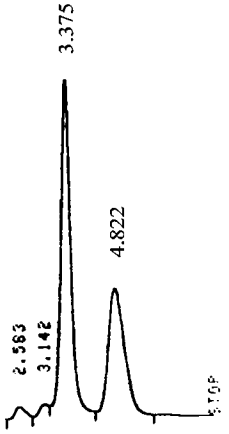
1.(b) iii



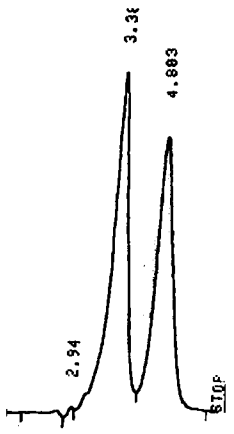
1.(b) iv



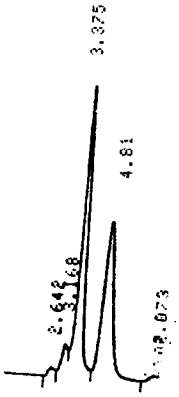
1.(b) v



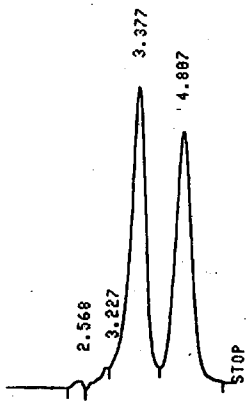
1.(b) vi



1.(b) vii



1.(b) viii



1.(b) ix

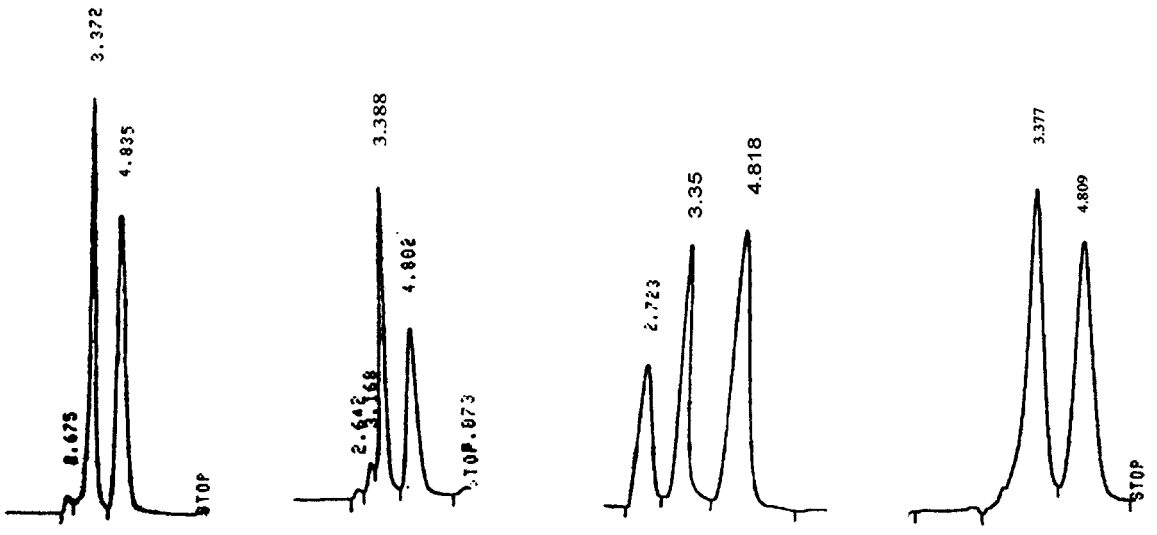


Figure 1. (a) HPLC chromatogram of metamitron (technical grade) and its metabolite. (b) HPLC chromatogram of metamitron and its metabolite, from cleanup extract from different tissues, urine, blood, and feces in acetonitrile/water (1:1, v/v): (i) blood; (ii) feces; (iii) urine; (iv) liver; (v) kidney; (vi) heart; (vii) fat; (viii) lung; (ix) adrenal gland; (x) skin; (xi) rumen; (xii) brain; (xiii) uterus.

Standard and samples (20 μL) were injected into the liquid chromatograph with the first and last being the standard.

Chromatographic Procedure. Stock solutions of metamitron and its metabolite [3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one] (100 ppm) were prepared in acetonitrile as standards. The retention times of metamitron and metabolite were 4.8 and 3.36 min, respectively (**Figure 1**). The retention times of parent and metabolite occurring in blood/feces/tissue/urine were compared with that of the external standard, and the data were recorded in an HP 3392A integrator.

Measurement of Cytochrome P_{450} Contents. Cytochrome P_{450} contents of microsomal pellets were estimated according to the methods of Omura and Sato (*8*). Microsomes were diluted with phosphate buffer (0.1 M, pH 7.4) to a protein concentration of 7.5 mg mL^{-1}. A volume of 1 mL of microsomal sample was taken in a cuvette, which then was reduced with sodium dithionite, and a baseline was generated between 400 and 500 nm using a double-beam UV−vis spectrophotometer (Chemitto, Spectrascan 2600). The sample was then bubbled with carbon monoxide (CO) for 45 s. A final scan produced the CO-treated minus reduced difference spectrum from which cytochrome P_{450} content was calculated using an extinction coefficient of 91 mM^{-1} for $A_{409-490nm}$.

Recovery. The recoveries of metamitron and its metabolite [3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one] were estimated by fortifying different substrates with known quantities to give final concentration of 0.5, 1.0, 2.0, and 4.0 ppm for blood, urine, and bile and 1.0, 2.0, 4.0, and 8 ppm for feces, tissues, and gastrointestinal contents. The area under HPLC peaks was plotted against several concentrations of metamitron and its metabolite, and linearity was found to be maintained. The linearity of the calibration curve was checked. The recoveries of metamitron and its metabolite from blood, urine, and other substrates ranged from 90 to 98%. The limit of detection for metamitron and its metabolite [3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one] was found to be 0.1 ppm.

Statistical Analysis of Data. Statistical analysis of data was conducted by using standard formulas (*9*).

RESULTS

Metamitron in Blood. The initial concentration of metamitron in blood at 5 min was found to be 12.0 ± 0.87 μg mL^{-1}. The maximum concentration was detected at 4 h (84.3 ± 8.60 μg mL^{-1}), and thereafter the concentration declined until 36 h. Metamitron could not be detected in the blood sample of goat at 48 h (**Figure 2**). The K_a, $t_{1/2}K_a$, β, $t_{1/2}\beta$, Vd_{area}, and AUC of metamitron were 0.69 ± 0.09 h^{-1}, 1.01 ± 0.12 h, 0.06 ± 0.00 h^{-1}, 8.98 ± 0.70 h, 2.00 ± 0.08 L kg^{-1}, and 1807 ± 103 μg h mL^{-1} respectively, and Cl_B, Cl_R,Cl_H, K_{12}, and K_{21} values were 0.15 ± 0.00 L kg^{-1} h^{-1}, 0.10 ± 0.00 L kg^{-1} h^{-1}, 0.05 ± 0.00 L kg^{-1} h^{-1}, 0.28 ± 0.04 h^{-1}, and 0.41 ± 0.03 h^{-1}, respectively (**Table 1**).

Table 1. Kinetic Parameters for Metamitron[a] after a Single High-Dose Oral Administration to Goats at 278 mg kg^{-1} (n = 6; Mean ± SE[b] Values for both Males and Females)

parameter	value	parameter	value
K_a (h^{-1})	0.69 ± 0.09	Cl_R (L kg^{-1} h^{-1})	0.10 ± 0.00
$t_{1/2}K_a$ (h)	1.01 ± 0.12	Cl_H (L kg^{-1} h^{-1})	0.05 ± 0.00
β (h^{-1})	0.06 ± 0.00	F_c	0.55 ± 0.01
$t_{1/2}\beta$ (h)	8.98 ± 0.70	K_{el} (h^{-1})	0.14 ± 0.00
Vd_{area} (L kg^{-1})	2.00 ± 0.08	K_{12} (h^{-1})	0.28 ± 0.04
AUC (μg h mL^{-1})	1807 ± 103	K_{21} (h^{-1})	0.41 ± 0.03
Cl_B (L kg^{-1} h^{-1})	0.15 ± 0.00	T ∼ B	0.80 ± 0.04

[a] Test chemical. [b] SE, standard error.

Recovery of Metamitron and Its Metabolite. *Feces.* Excretion of metamitron and its metabolite was recorded at 24 h, and maximum quantity of both parent and metabolite was excreted at 24 h. The levels decreased to below the detection limit by 96 h for the parent and by 144 h for the metabolite (**Table 2**).

Urine. Excretion of maximum quantity of metamitron through urine was recorded at 24 h. By 72 h, the level of parent was below the detection limit. Metabolite was excreted through urine within 24 h, reached a maximum in the 48 h urine sample, and by 144 h the level was below the detection limit (**Table 3**).

Gastrointestinal Tract Contents. The maximum quantity of metamitron was recovered from rumen content on days 4−6 in sequence followed by large (except for the day 6 sample) and small intestinal contents. The maximum quantity of metabolite was recovered from small intestinal content on days 4−7 followed by large intestinal content and rumen content in sequence (**Table 4**).

Tissue. The mean residual contents of metamitron and its metabolite recovered from different tissues of goats are presented in **Table 5**. The maximum quantity of parent compound and its metabolite was recovered from goats on days 4 and 5,

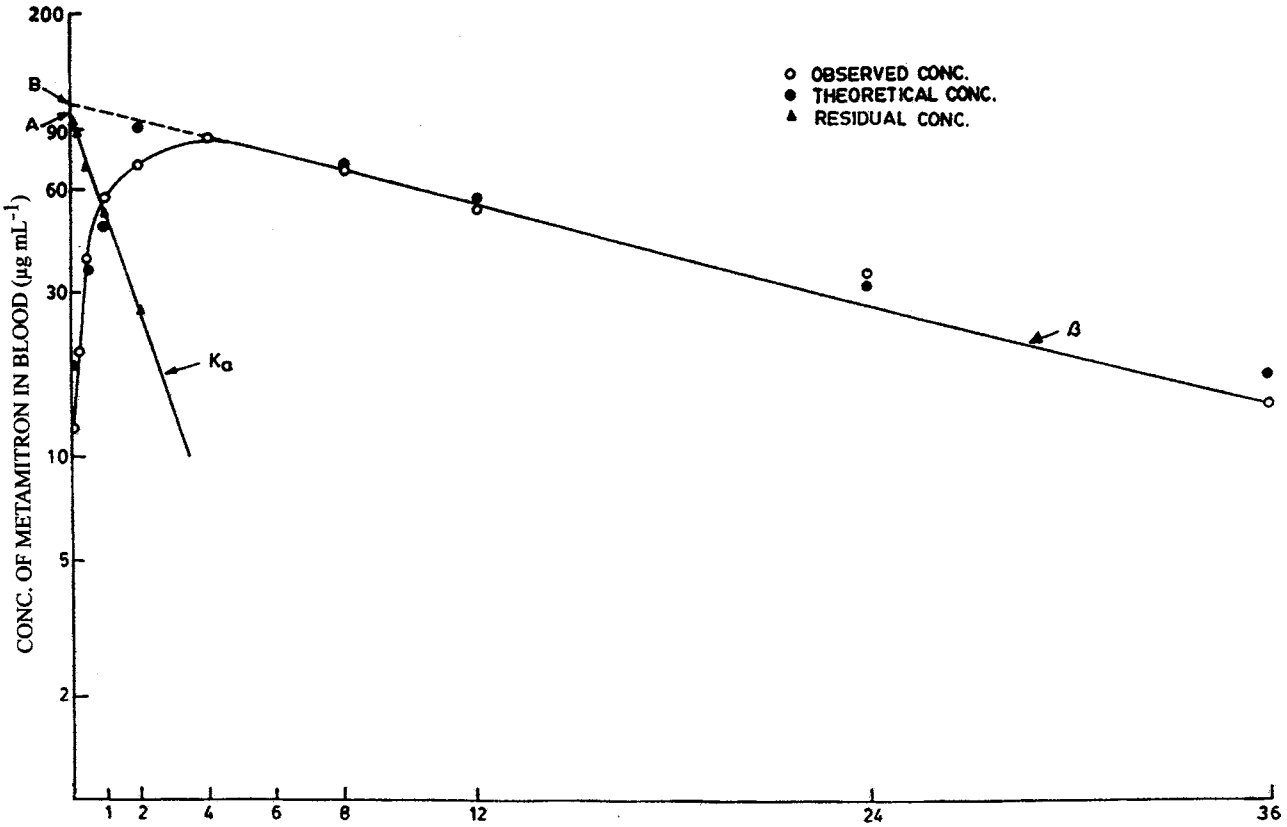


Figure 2. Semilogarithmic plot of mean blood concentration of metamitron (μg mL^{-1}) against time following a single oral high dose at 278 mg kg^{-1} in goats.

Table 2. Metamitron and Its Metabolite Recovered from Feces (Parts per Million) of Goat following Oral Administration of a Single High Dose at 278 mg kg^{-1}[a]

	group of goat sacrificed on day							
	4		5		6		7	
time (h)	P	M	P	M	P	M	P	M
0–24	300 ± 29.6	1719 ± 163	153 ± 19.3	1662 ± 209	328 ± 34.0	604 ± 65.6	117 ± 14.7	523 ± 65.8
24–48	453 ± 26.5	1065 ± 62.4	348 ± 22.0	1343 ± 84.9	491 ± 39.6	487 ± 39.2	463 ± 26.0	563 ± 31.6
48–72	100 ± 10.2	791 ± 46.3	102 ± 6.85	1083 ± 68.4	105 ± 7.60	418 ± 33.6	160 ± 12.5	739 ± 64.2
72–96	64.4 ± 5.71	755 ± 81.2	86.0 ± 12.5	1079 ± 141	96.0 ± 7.13	588 ± 45.3	67.4 ± 5.93	737 ± 64.7
96–120	–	–	BDL	525 ± 47.2	BDL	419 ± 45.6	BDL	566 ± 71.6
120–144	–	–	–	–	BDL	307 ± 33.3	BDL	376 ± 33.0
144–168	–	–	–	–	–	–	BDL	263 ± 35.2

[a] Mean value of six replicates with SE values of males and females; BDL, below detection limit (<0.1 ppm); P, parent; M, metabolite; –, not applicable.

Table 3. Metamitron and Its Metabolite Recovered (Parts per Million) from Urine of Goat Following Oral Administration of a Single High Dose at 278 mg kg^{-1}[a]

	group of goat sacrificed on day							
	4		5		6		7	
time (h)	P	M	P	M	P	M	P	M
0–24	690 ± 45.5	396 ± 26.0	491 ± 29.3	505 ± 30.2	609 ± 50.3	337 ± 27.9	489 ± 38.0	191 ± 14.8
24–48	356 ± 14.7	1057 ± 43.7	132 ± 5.52	932 ± 38.8	345 ± 20.5	1273 ± 75.2	230 ± 15.0	609 ± 39.7
48–72	103 ± 9.88	682 ± 50.4	56.8 ± 3.78	889 ± 44.7	90.4 ± 5.45	310 ± 18.5	84.5 ± 5.60	457 ± 30.3
72–96	BDL	213 ± 15.6	BDL	271 ± 12.5	BDL	204 ± 10.2	BDL	243 ± 16.0
96–120	–	–	BDL	163 ± 8.18	BDL	122 ± 6.09	BDL	193 ± 12.7
120–144	–	–	–	–	BDL	66.5 ± 3.31	BDL	126 ± 8.36
144–168	–	–	–	–	–	–	BDL	BDL

[a] Mean value of six replicates with SE values of males and females; BDL, below detection limit (<0.1 ppm); P, parent; M, metabolite; –, not applicable.

respectively, after administration. The adrenal gland retained the highest concentration of metamitron residue on day 4 followed by bile, uterus, kidney, and reticulum in sequence. The small intestine retained the highest concentration of metabolite followed by heart, reticulum, and bile in sequence on day 4. Tissues such as the large intestine, small intestine,

Table 4. Metamitron and Its Metabolite Recovered (Parts per Million) from Gastrointestinal Contents of Goats Following Oral Administration of a Single High Dose at 278 mg kg^{-1}[a]

	group of goat sacrificed on day							
	4		5		6		7	
substrate	P	M	P	M	P	M	P	M
rumen content	98.4 ± 6.66	24.3 ± 1.82	32.5 ± 3.21	26.3 ± 2.64	7.25 ± 0.55	29.8 ± 2.48	BDL	37.8 ± 3.50
large intestinal content	24.1 ± 1.61	277 ± 20.7	6.20 ± 0.62	154 ± 17.4	4.10 ± 0.31	188 ± 17.6	BDL	264 ± 25.6
small intestinal content	11.7 ± 0.85	1001 ± 72.6	5.70 ± 0.36	785 ± 50.3	5.35 ± 0.40	823 ± 64.0	BDL	704 ± 54.6

[a] Mean value of six replicates with SE values of males and females; BDL, below detection limit (<0.1 ppm); P, parent; M, metabolite.

Table 5. Metamitron and Its Metabolite Recovered (Parts per Million) from Different Tissues of Goat Following Oral Administration of a Single High Dose at 278 mg kg^{-1}[a]

	group of goat sacrificed on day							
	4		5		6		7	
substrate	P	M	P	M	P	M	P	M
liver	2.85 ± 0.25	10.3 ± 0.91	BDL	7.04 ± 0.79	BDL	10.4 ± 1.16	BDL	3.76 ± 0.66
kidney	24.0 ± 2.40	29.8 ± 3.40	12.1 ± 1.37	22.7 ± 2.50	20.2 ± 0.75	15.2 ± 1.50	BDL	BDL
heart	5.57 ± 0.42	41.5 ± 3.14	2.10 ± 0.07	31.0 ± 3.40	1.33 ± 0.13	10.0 ± 1.66	BDL	BDL
lung	10.6 ± 1.13	6.93 ± 0.73	BDL	4.72 ± 0.48	BDL	BDL	BDL	BDL
muscle	BDL	7.49 ± 0.79	BDL	5.41 ± 0.52	BDL	2.66 ± 0.18	BDL	BDL
spleen	10.5 ± 1.00	36.5 ± 3.50	BDL	7.20 ± 0.40	BDL	10.0 ± 0.33	BDL	BDL
fat	5.75 ± 0.40	12.4 ± 0.86	4.86 ± 0.28	19.4 ± 1.15	15.3 ± 1.72	4.54 ± 0.54	BDL	0.85 ± 0.00
adrenal gland	50.0 ± 5.00	BDL	7.04 ± 0.70	BDL	BDL	BDL	BDL	BDL
brain	BDL	19.3 ± 1.87	BDL	26.8 ± 2.49	BDL	8.75 ± 0.87	BDL	BDL
uterus (3)	30.8 ± 7.42	9.42 ± 1.99	BDL	7.75 ± 1.75	BDL	10.0 ± 1.00	BDL	BDL
ovary (3)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
testis (3)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
bile	33.3 ± 3.33	40.0 ± 3.33	BDL	28.8 ± 2.22	BDL	25.0 ± 1.92	BDL	24.0 ± 2.40
skin	19.1 ± 1.93	24.6 ± 2.48	6.72 ± 0.50	23.8 ± 1.80	2.24 ± 0.19	22.0 ± 2.87	BDL	24.4 ± 4.19
large intestine*	7.30 ± 0.80	7.80 ± 0.70	4.84 ± 0.48	7.00 ± 0.68	5.11 ± 0.11	7.22 ± 0.72	2.15 ± 0.21	4.68 ± 0.10
small intestine*	7.89 ± 0.89	70.4 ± 7.96	3.71 ± 0.59	41.5 ± 6.61	4.11 ± 0.26	53.1 ± 3.07	1.34 ± 0.07	50.6 ± 6.93
rumen*	17.2 ± 1.85	30.0 ± 3.62	8.45 ± 0.31	14.2 ± 2.25	8.42 ± 0.57	8.07 ± 0.73	1.62 ± 0.03	BDL
reticulum*	22.8 ± 4.20	41.4 ± 7.60	10.2 ± 1.33	20.8 ± 2.66	13.8 ± 0.18	18.0 ± 0.20	5.76 ± 0.17	BDL
omasum*	11.2 ± 1.00	6.20 ± 0.40	4.66 ± 0.22	3.11 ± 0.22	4.50 ± 0.25	5.00 ± 0.27	1.92 ± 0.13	BDL
abomasum*	11.1 ± 1.20	10.4 ± 1.10	4.27 ± 0.11	6.72 ± 0.88	6.00 ± 0.23	5.88 ± 0.23	2.30 ± 0.10	BDL
bone	0.62 ± 0.03	16.1 ± 0.94	0.33 ± 0.00	13.2 ± 1.57	0.45 ± 0.01	18.5 ± 0.64	0.19 ± 0.00	15.8 ± 0.94
mammary gland (3)	BDL	6.50 ± 0.25	BDL	2.60 ± 0.20	BDL	BDL	BDL	BDL
blood	BDL	19.0 ± 2.86	BDL	7.74 ± 0.89	BDL	10.3 ± 1.18	BDL	BDL

[a] Mean value of six replicates with SE values of males and females; BDL, below detection limit (<0.1 ppm); P, parent; M, metabolite; number in parentheses indicates number of goats; *, content not included.

Table 6. Total Recovery Percentage of Metamitron and Its Metabolite from Goats Sacrificed on Different Days Following Oral Administration of a Single High Dose at 278 mg kg^{-1}[a]

	day 4		day 5		day 6		day 7	
substrate	P	M	P	M	P	M	P	M
feces	3.42 (152)	17.1 (761)	2.77 (161)	23.4 (1371)	6.13 (196)	17.9 (573)	4.32 (156)	21.1 (765)
gastrointestinal content	5.52 (245)	9.33 (415)	1.64 (96.7)	8.60 (502)	0.52 (16.5)	9.45 (330)	–	9.56 (346)
urine	13.7 (608)	26.4 (1175)	7.03 (410)	28.1 (1643)	12.3 (395)	23.3 (744)	10.0 (364)	24.2 (878)
tissues	0.88 (38.9)	3.85 (170)	0.35 (20.6)	2.93 (175)	0.31 (10.0)	2.47 (79.9)	0.05 (2.10)	0.18 (49.9)
recovery %	23.5	56.6	11.7	63.0	19.2	53.1	14.3	55.0
recovery	80.1		74.7		72.3		69.3	

[a] Figure in parentheses indicates total milligrams recovered; P, parent; M, metabolite; –, not applicable.

rumen, reticulum, omasum, abomasum, and bone retained metamitron on day 7. Only the small intestine retained the highest level of metabolite on day 7 followed by skin, bile, bone, liver, and fat in sequence.

Total Recovery Percentage. Maximum percentages of parent and its metabolite in terms of parent compound were recovered from urine samples. Total recovery percentages from feces, gastrointestinal contents, urine, and tissues for metamitron and its metabolite in terms of parent compound from groups of goats on days 4, 5, 6, and 7 after dosing were 80.1, 74.7, 72.3, and 69.3, respectively (**Table 6**).

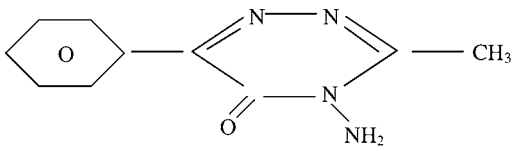
Metabolism in Goat. One metabolite, namely, 3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one, was detected in urine, feces, gastrointestinal content, and tissues. The concentration of recovered metabolite was highest in urine, followed by concentrations in feces, gastrointestinal content, and tissues.

Microsomal Study. Mean cytochrome P_{450} contents of liver microsomal pellets of groups 1 and 2 were 1.65 ± 0.08 and 2.70 ± 0.09 nmol mg^{-1} of microsomal protein, respectively. Metamitron-treated goats showed a significant ($p < 0.01$) increase of cytochrome P_{450} contents of liver microsomal pellet (**Table 7**).

Table 7. Effect of Metamitron on Cytochrome P_{450} Contents of Liver Microsomal Pellet of Goats Following Repeated Oral Administration at 30 mg kg^{-1} for 7 Days[a]

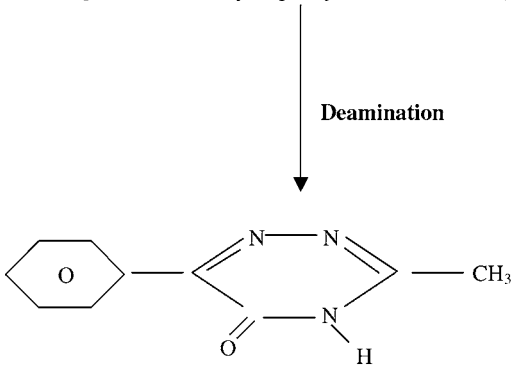
	animal					
	1	2	3	4	mean with SE	*F* value
group 1 (control)	1.65	1.72	1.44	1.82	1.65 ± 0.08	15.2*
group 2	3.21	2.33	3.06	2.20	2.70 ± 0.09	

[a] Cytochrome P_{450} results reported as nanomoles per milligram of microsomal protein; *, $p < 0.01$ between groups 1 and 2; SE values of male and female goats.



Metamitron

[4-amino-3-methyl-6-phenyl-1,2,4 – triazin-5 (4H)-one]



Metabolite

[3-methyl-6-phenyl-1,2,4 – triazin-5 (4H)-one]

Figure 3. Probable metabolic pathway of metamitron.

DISCUSSION

Detection of herbicide in the 0.08 h blood sample coupled with the absorption rate constant (K_a) value indicates that metamitron is rapidly absorbed from the gastrointestinal tract of the goat. The values of the tissue/blood ratio (T ∼ B) and the fraction of herbicide in the central compartment (F_c) indicate that metamitron after attaining pseudoequilibrium may be retained either in blood or in tissue or in both. Elimination half-life ($t_{1/2}\beta$), low body clearance (Cl_B) value, and K_{21} value (transfer of compound from tissue to blood) suggest that the compound may be retained in the blood, and consequently it was detected in the blood until 36 h after a single oral administration. The maximum residue of parent compound was detected in the adrenal gland followed by bile on day 4 of sacrifice. Metamitron has little tendency to accumulate in the tissue because a very small amount of parent compound varying from 0.05 to 0.88% was retained in the tissue. The apparent volume of distribution (Vd_{area}) value of the toxicokinetic study was 2.00 ± 0.08 L kg^{-1}, indicating wide distribution of herbicide to all parts of the body, and in accordance with the observation, all of the goat tissues except muscle, ovary, testis, and mammary gland on day 4 after a single oral administration retained the residue, whereas only bone and gastrointestinal contents retained the residue on day 7 after administration. The renal clearance (Cl_R) value is 1.8 times greater than the hepatic clearance (Cl_H) value, and in consonance 34−40% of metamitron including its metabolite against the administered dose was recovered from urine, of which the metabolite in terms of parent compound contributed >65%. Likewise, 20−26% of the herbicide including its metabolite was recovered from feces of goat at different days of sacrifice, whereas metabolite in terms of parent compound was >74%. Similarly, metabolite in terms of parent compound contributed 81% of the recovery made from the tissues of goats sacrificed at different days after administration. Metamitron caused induction of the cytochrome P_{450} content of liver tissue of goats. It appears that metamitron undergoes oxidative deamination very rapidly, yielding the metabolite [3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one], and a tentative metabolic pathway was proposed showing oxidative deamination of metamitron (**Figure 3**). Material balance of the herbicide including metabolite in terms of parent compound after a single oral administration (69.3−80.1%) seems to be quite satisfactory.

ACKNOWLEDGMENT

We acknowledge the gift of technical grade metamitron and its metabolite [3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one] from M/S Gharda Chemicals Ltd., Mumbai, India.

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Received for review May 23, 2003. Revised manuscript received July 20, 2003. Accepted July 28, 2003.

JF034541U