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Toxicokinetics, Recovery, and Metabolism of Triclopyr Butotyl (ACTP) Ester in Goats

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Toxicokinetic behavior, recovery, and metabolism studies of ACTP ester and its effect on cytochrome P_{450} content of liver microsomal pellet were carried out in black Bengal goat after a single intravenous administration of 11.88 mg kg^{-1} and consecutive oral administration of 79.22 mg kg^{-1} for 7 days. ACTP ester achieved a maximum blood concentration of 42.64 ± 4.26 μg mL^{-1} at 0.08 h after intravenous administration followed by a sharp decline until 0.5 h, and the minimum blood concentration was recorded at 36 h (1.93 ± 0.14 μg mL^{-1}) postdosing. The kinetic behavior of ACTP ester followed a "two-compartment open model". Comparatively shorter α (0.81 ± 0.02 h^{-1}) and greater $t_{1/2(\alpha)}$ (0.86 ± 0.03 h) indicated a slower rate of distribution of ACTP ester in goat. The $t_{1/2(\beta)}$ (14.83 ± 1.49 h) and $V_{d(area)}$ (0.91 ± 0.19 L kg^{-1}) suggested a longer elimination phase with general distribution in all compartments of the body. The higher T/B and K_{12}/K_{21} values associated with a lower f_c value suggested longer persistence in the tissue compartment at higher concentration. The higher Cl_R compared to Cl_H indicated the major amount was eliminated by the kidney. Maximum concentration of ACTP ester including its metabolites, triclopyr acid and trichloropyridinol, was excreted through urine at 48 h. The recovery of ACTP ester including metabolites after repeated nontoxic oral dose administration was 70.09%, of which recovery from feces was 4.45%, suggesting the major portion of administered ACTP ester was absorbed through the gastrointestinal tract of the goat. All of the tissues contained ACTP ester and its metabolites. ACTP ester did not alter the cytochrome P_{450} content of the liver tissue following repeated nontoxic oral dose administration for 7 days.

KEYWORDS: Toxicokinetics; recovery; ACTP ester; metabolites; cytochrome P_{450}; goat

INTRODUCTION

Triclopyr butotyl (ACTP) ester is a herbicide structurally related to picloram and widely used to control weeds. It is rapidly hydrolyzed to triclopyr acid in plant systems, which then is rapidly absorbed by the foliage and roots by the process of translocation throughout the plant accumulating in meristamatic tissues and inducing auxin-type responses in susceptible species (mainly broad-leafed weeds and grass weeds) (*1*). No residue of triclopyr could be detected in milk or feces after oral feeding in a lactating cow (*2*). Necessary data with respect to toxicokinetics, retention in tissues, and identification and quantification of metabolites of the herbicide need to be generated in animal systems before it is widely used and declared safe to avoid possible public health hazards. The goat is considered to be a good choice of experimental animal because its meat and milk are widely accepted by human beings. Literature relating to toxicokinetics and metabolism of ACTP ester is not available in animals, particularly in black Bengal goats. With this idea, the present experiment studied the intravenous toxicokinetics, metabolism, and recovery following repeated nontoxic oral dose administration of ACTP ester in black Bengal goats.

EXPERIMENTAL PROCEDURES

Chemicals. ACTP ester (analytical grade, purity = 97%) and its metabolites, triclopyr acid (purity = 97.2%) and 3,5,6-trichloro-2-pyridinol (purity = 97.5%), were supplied by M/S Gharda Chemicals Ltd., Mumbai, India. These compounds were further purified and authenticated by HPLC and spectroscopic (UV, IR, MS, and NMR) analysis. All of the chemicals used in this study were obtained from E. Merck and Sigma Chemical Co.

Animal Treatment. Clinically healthy black Bengal male and female (nulliparous) goats of approximately 1.5−2 years of age, weighing from 9.5 to 12 kg, were used in this experiment. Throughout the period of

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experiment, the animals were caged individually in custom-made metabolic cages (stainless steel). The temperature of the experimental animal room was maintained at 22 °C (± 3 °C) and artificial lighting facilities, ad libitum drinking water, and standard feed were provided (*3*). Each animal was fasted overnight before administration of vehicle and or ACTP ester. For the intravenous toxicokinetic study, four male and four female goats were used, of which one male goat and one female goat were kept as controls. The remaining three male goats and three female goats were considered to be the experimental animals. ACTP ester at 11.88 mg kg^{-1} dissolved in 1 mL of glycerinformal was administered intravenously (iv) through the jugular vein to each goat of the experimental group. The same amount of glycerinformal was administered intravenously to each goat of the control group.

For the total recovery study, eight clinically healthy goats (four males and four females) were used. Among them, one male and one female were kept as controls and the remaining six goats were considered to be the experimental group. Goats of the experimental group were dosed orally with ACTP ester at 79.22 mg kg^{-1} suspended in 25 mL of carboxymethylcellulose (1% CMC) once daily for 7 consecutive days.

For the microsomal study six male and six female goats were considered and divided into three equal groups, each having two male and two female goats.

Fixation of Single Minimum Intravenous Toxic Dose. For determination of the minimum intravenous toxic dose level of ACTP ester, four male and four female goats were divided into four groups each containing one male and one female. Three different dose levels of ACTP ester, that is, 2.97, 5.94, and 11.88 mg kg^{-1} [1/240, 1/120, and 1/60 of the LD_{50} of triclopyr acid (713 mg kg^{-1})] (*4*) were administered intravenously to the three different groups separately. The remaining group was considered to be the control and received glycerinformal. Goats treated with ACTP ester at 2.97 and 5.94 mg kg^{-1} did not exhibit any sign of toxicity, whereas goats treated with 11.88 mg kg^{-1} produced signs of toxicity without causing mortality during the observation period. The toxic symptoms include depression and drowsiness after 10 min, miosis and fixation of the eyelid, increased secretion of nasal discharge and salivation, irregular skin itching, yawning, muscle tremors mainly on the posterior portion of the body, slight increase of temperature, and increased frequency of defecation until 4.30 h after administration. Accordingly, ACTP ester at 11.88 mg kg^{-1} was considered the minimum intravenous toxic dose.

Determination of Maximum Nontoxic Oral Dose. The objective is to find a dose level of ACTP ester that on single oral administration for 7 consecutive days would not cause any toxic symptoms. For fixation of oral dose level of ACTP ester, eight goats (four males and four females) were divided into four equal groups containing one male and one female in each group. ACTP ester at three different doses of 59.41, 79.22, and 101.85 mg kg^{-1} [1/12, 1/9, and 1/7 of the reported LD_{50} of triclopyr acid (713 mg kg^{-1})] dissolved in 1% CMC was administered orally once daily to each animal of the different groups of goats for 7 consecutive days, respectively. The remaining group was considered to be the control and received only the same volume of 1% CMC. Goats treated with ACTP ester at 59.41 and 79.22 mg kg^{-1} did not exhibit any physical signs of toxicity, whereas the goats administered 101.85 mg kg^{-1} showed grinding of teeth and rubbing of skin immediately after administration, which then waned out after 1 h of administration on each day. In addition, goats defecated semisolid stool and showed generalized depression from the fifth day onward and returned to normal after cessation of administration. The control group of goats fed with 1% CMC every day for 7 consecutive days did not show any sign of toxic symptoms. Therefore, the dose level of 79.22 mg kg^{-1} was selected as the maximum oral nontoxic dose.

Kinetics. For kinetic study, blood samples were collected from the jugular vein of each experimental goat into heparinized tubes at different time intervals (0.08, 0.16, 0.25, 0.33, 0.50, 0.67, 1, 2, 3, 4, 6, 8, 12, 24, 36, 60, 72, and 96 h) after ACTP ester administration in two sets of test tubes separately. Concentrations of ACTP ester and its two metabolites (triclopyr acid and trichloropyridinol) were estimated by HPLC. The values of kinetic parameters such as C_B^0, α, $t_{1/2}$ (α), β, $t_{1/2}$ (β), T/B, $V_{d,area}$, $V_{d,B}$, $V_{d,c}$, $V_{d,ss}$, f_c, AUC, K_{el}, K_{12}, K_{21}, K_{12}/K_{21}, Cl_B, Cl_R. and Cl_H were determined from computerized (Pharmkit, supplied by Department of Pharmacology, JIPMER, Pondichery, India) semilogarithmic plots of blood level time profile data in goats using standard formulas (*5*).

Blood Concentration of ACTP Ester during Repeated Administration. Blood samples (1 mL) were collected from the jugular vein into heparinized tubes from each experimental goat 4 h after oral administration followed every 24 h before successive administration of ACTP ester until 144 h.

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

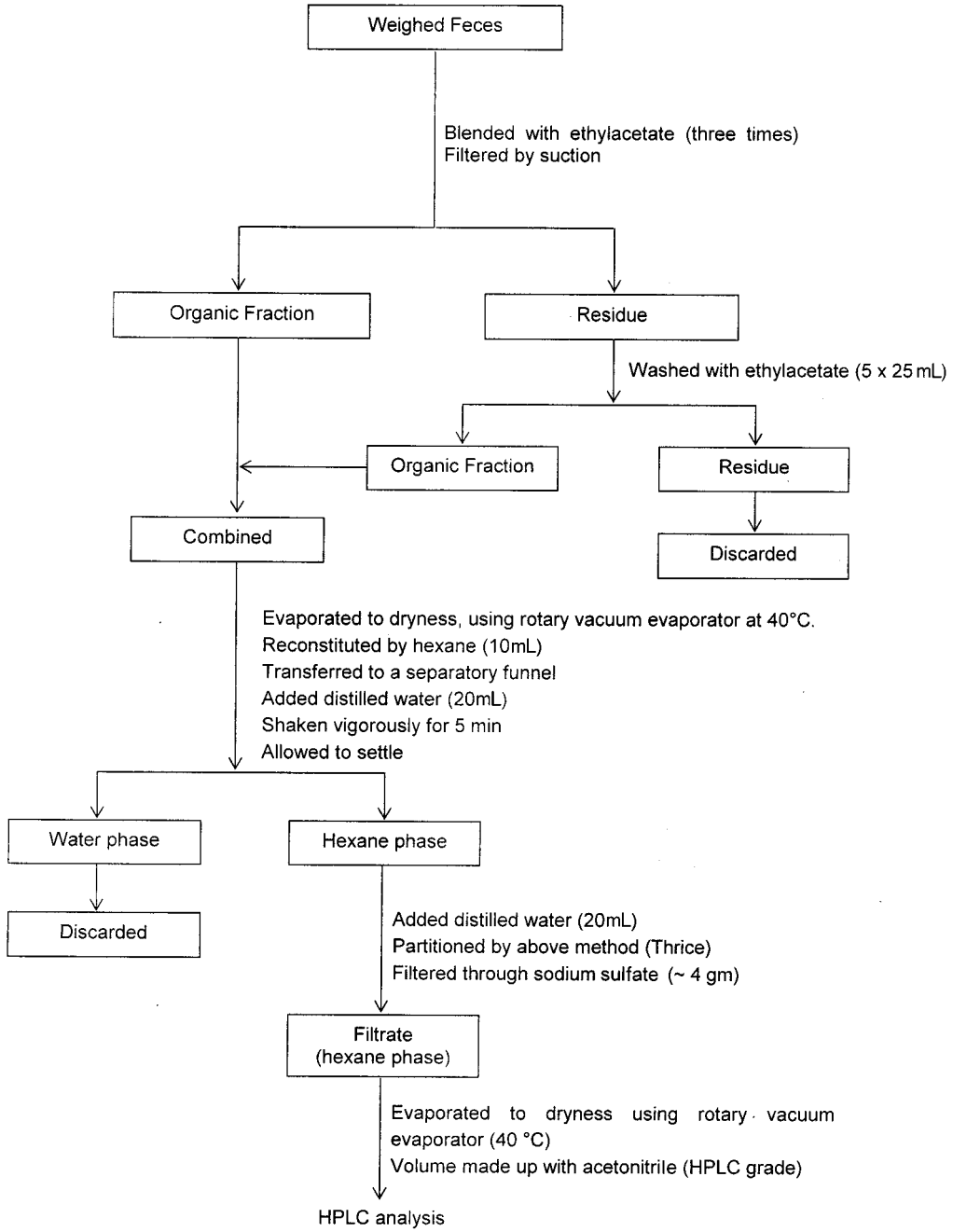
For total recovery study, following repeated nontoxic oral dose administration, urine and feces from individual goat were collected at 24, 48, 72, 96, 120, 144, and 168 h. The samples were measured or weighed and stored at −20 °C prior to extraction.

Collection of Tissues, Bile, and Gastrointestinal Contents. The animals were slaughtered on the eighth day after repeated nontoxic oral dosing of ACTP ester for 7 consecutive days and liver, kidney, lungs, brain, heart, spleen, adrenal gland, omental fat, ovary, uterus, testis, rumen, reticulum, omasum, abomasum, intestine, bile, contents of rumen and intestine, and samples of thigh muscle, bone, and skin were taken, weighed, minced, and stored at −20 °C prior to extraction. A sample (2 g) of each of the above-mentioned tissues except bile (5 mL) was extracted with respective solvent, and ACTP ester and its metabolites 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, quantified, and multiplied with factors as described by Davis et al. to get the total recovery (*6*).

Microsomal Study. ACTP ester at 79.22 mg kg^{-1} suspended in CMC (1% w/v) was administered orally once daily for 7 consecutive days to each goat of group 2, but only CMC was administered orally in the aforesaid procedure to each goat of group 1 (control). Phenobarbitone sodium (Gardenal) at 60 mg kg^{-1} in distilled water was administered orally once daily to each goat of group 3 for 5 continuous days. Group 3 goats were killed on day 6, and group 1 and 2 goats were sacrificed on day 8 of administration. Pieces of liver (caudate lobe) were taken, trimmed of debris, minced, and washed with ice-cold 1.15% KCl within 10 min, and 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 127A). The homogenate was centrifuged at 10^4 in an automatic high-speed refrigerated centrifuge (SCR 20B, rotor RPR 20-2) for 30 min, and the supernatant was recentrifuged at 105000*g* for 1 h in a Microultracentrifuge (Hitachi CS-120 GX, rotor $S100AT_4$) to yield the microsomal pellet. The latter was suspended in buffer [potassium pyrophosphate (0.1 M, pH 7.4) containing ethylenediaminetetraacetic acid (10 mM) and butylated hydroxytoluene (20 μM)] and homogenized with four passes through a mechanically driven Teflon−glass homogenizer and again centrifuged at 105000*g* for 1 h. The supernatant fraction was decanted, the microsomal pellet was resuspended in a minimum volume of buffer [tris(hydrochloride) (10 mM, pH 7.4), ethylenediaminetetraacetic acid (10 mM), and glycerol 200 mL L^{-1}], and cytochrome P_{450} was immediately measured. Protein was measured according to the colorimetric method (*7*).

Extraction and Cleanup. *Urine.* To each urine sample (10 mL) was added and mixed distilled water (25 mL), and the mixture was transferred to a dry clean separatory funnel (125 mL). Hexane (40 mL) was then added, shaken vigorously for 5 min, and allowed to settle for 2−3 min until the hexane and urine phases were distinctly separated. The lower urine phase was again transferred to another separatory funnel, and the above-said procedure was repeated twice. Cumulative upper hexane phase was passed through sodium sulfate (~4 g) and evaporated to dryness using a rotary vacuum evaporator at 40 °C; the residue was dissolved in acetonitrile (HPLC grade) for subsequent HPLC analysis.

Scheme 1. Extraction and Analysis of Caprine Feces



Feces. The method of extraction from feces and intestinal and ruminal contents is presented in **Scheme 1**.

Blood and Bile. To each sample of blood (1 mL) or bile (5 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 hexane (20 mL), and only the organic layer was filtered through sodium sulfate (~4 g). The test tubes were washed three times with hexane (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.

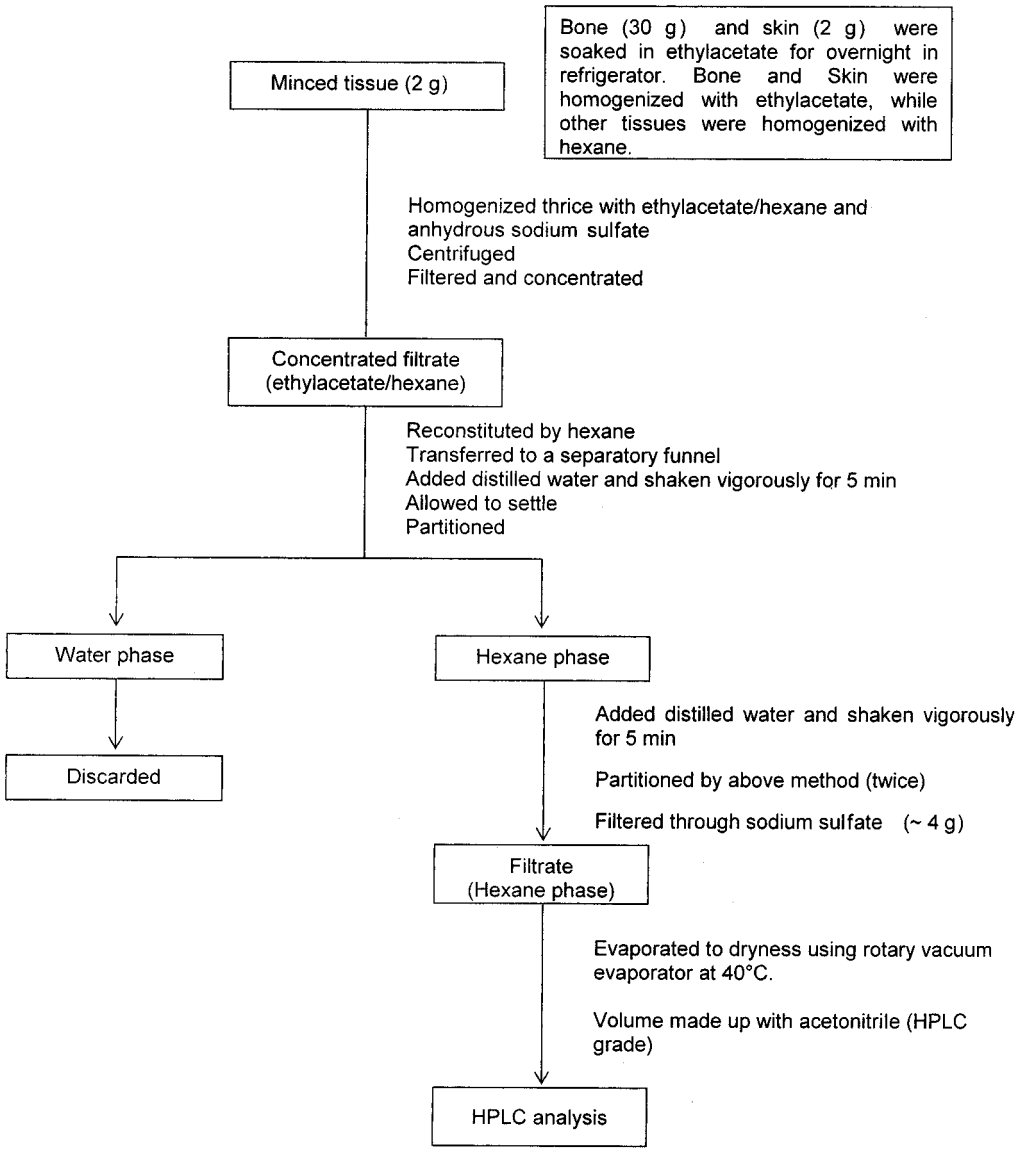
Tissues. The method of extraction and cleanup of tissue samples is presented in **Scheme 2**. A small quantity of minced tissue (2 g) except bone and skin was homogenized with hexane (20 mL), whereas crushed bone (30 g) and minced skin (2 g) were soaked in ethyl acetate (10 mL) and kept overnight in 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 ACTP ester and its metabolites with the following operational parameters: mobile phase, acetonitrile/water (9:1), the mixture was subject to membrane filtration and degassed by ultrasonification; λ value, 280 nm; column, RPC-18 cartridge column; flow rate, 0.5 mL min^{-1}; injection using 25 μL loop with a Hamilton syringe. Standard and samples (20 μL) were injected into the liquid chromatograph with the first and last being the standard.

Chromatographic Procedure. Stock solutions of ACTP ester and its two metabolites, triclopyr acid (TCPA) and trichloropyridinol (TCP) (100 ppm), were prepared in hexane as standards. The retention times of ACTP ester, triclopyr acid, and trichloropyridinol were 3.64, 2.3, and 2.8 min, respectively (**Figure lA**). The retention times of the parent and metabolites occurring in blood/tissue/feces/urine were compared with that of the external standard, and the data were recorded in an HP 3392 A integrator.

Measurement of Cytochrome P_{450} Contents. Cytochrome P_{450} contents of the microsomal pellet 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

Scheme 2. Extraction and Analysis of Caprine Tissue, Bone, and Skin



mL^{-1}. A volume of 1 mL of microsomal sample was taken in a cuvette, which then was reduced with sodium dithionite, and a new baseline was generated between 400 and 500 nm using a Shimadzu UV 265 dual-beam spectrophotometer. The sample was then bubbled with 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} cm^2 for $A_{409-490nm}$.

Statistical Analysis. Statistical analyses of data were carried out (*9*).

RESULTS

Recovery. The recoveries of ACTP ester and its metabolites were estimated by fortifying different substrates with known quantities to give final concentrations of 0.5, 1.0, 2.0, and 4.0 ppm for blood, urine, and bile and 1.0, 2.0, 4.0, and 8.0 ppm for feces, tissues, and gastrointestinal contents. The limits of detection for ACTP ester, triclopyr acid, and trichloropyridinol were 0.07, 0.06, and 0.09 ppm, respectively. The linearity for all of the compounds was checked by calibration curve. The percentage of recovery from different substrates varied from 85.2 to 93.7.

Intravenous Study. ACTP Ester in Blood. The maximum concentration of ACTP ester in blood was found to be at 0.08 h (42.64 ± 4.26 μg mL^{-1}), and thereafter the concentration declined until 36 h (1.93 ± 0.14 μg mL^{-1}) after a single intravenously administered dose (**Table 1**). The kinetic behavior followed a "two-compartment open model" (**Figure 2**). The α, $t_{1/2(\alpha)}$, β, and $t_{1/2(\beta)}$ of ACTP ester were 0.81 ± 0.02 h^{-1}, 0.86 ± 0.03 h, 0.05 ± 0.004 h^{-1}, and 14.83 ± 1.49 h, respectively. The $V_{d,area}$, $V_{d,c}$, $V_{d,ss}$, and $V_{d,B}$ were 0.91 ± 0.19, 0.19 ± 0.02,

Table 1. Blood Level of ACTP Ester after Administration of a Single Intravenous Dose at 11.88 mg kg^{-1} in Goats (n = 6, Mean ± SE Values of both Male and Female)

time (h)	ACTP ester (ppm)	time (h)	ACTP ester (ppm)
0.08	42.64 ± 4.26	6.00	8.44 ± 1.18
0.16	33.94 ± 3.81	8.00	7.65 ± 1.04
0.25	26.42 ± 3.32	12.00	6.39 ± 0.69
0.33	22.88 ± 3.22	24.00	3.41 ± 0.32
0.50	16.42 ± 2.57	36.00	1.93 ± 0.14
0.67	12.03 ± 2.13	48.00	BDL[a]
1.00	10.87 ± 1.44	60.00	BDL
2.00	10.42 ± 1.51	72.00	BDL
3.00	9.85 ± 1.46	96.00	BDL
4.00	9.22 ± 1.36		

[a] BDL, below detection limit.

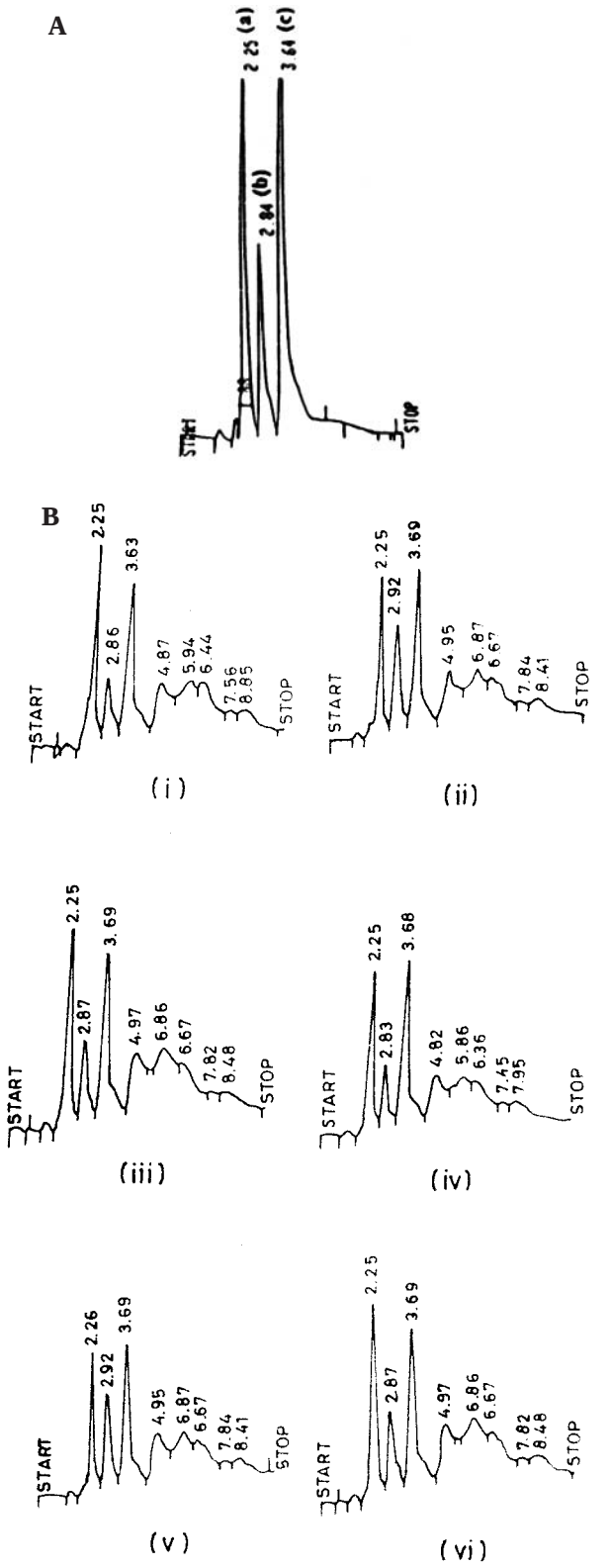


Figure 1. (A) Chromatogram of (a) triclopyr acid, (b) trichloropyridinol, and (c) ACTP ester (analytical grade). (B) HPLC chromatograms of ACTP ester and its metabolites, triclopyr acid and trichloropyridinol, from cleanup extract from different tissues, urine, and feces in acetonitrile/water (9:1 v/v): (i) brain; (ii) lung; (iii) urine; (iv) kidney; (v) feces; (vi) adrenal gland.

0.72 ± 0.14, and 1.18 ± 0.26 L kg^{-1}, respectively, and T/B, f_c, K_{el}, K_{12}, K_{21}, Cl_B, Cl_R, Cl_H, and AUC were 3.55 ± 0.54, 0.23 ± 0.02, 0.21 ± 0.02 h^{-1}, 0.46 ± 0.02 h^{-1}, 0.19 ± 0.02 h^{-1}, 0.04 ± 0.004 L kg^{-1} h^{-1}, 0.03 ± 0.004 L kg^{-1} h^{-1}, 0.009 ± 0.0006 L kg^{-1} h^{-1}, and 299.88 ± 25.50 μg h^{-1} mL^{-1}, respectively (**Table 2**).

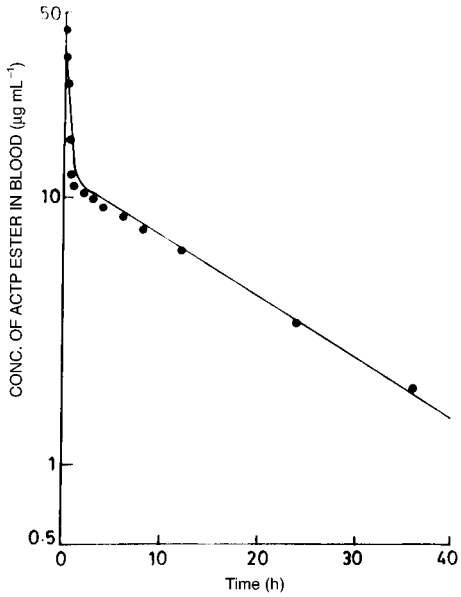


Figure 2. Semilogarithmic plot of mean blood concentration of ACTP ester against time with computerized best-fit line after administration of a single intravenous dose at 11.88 mg kg^{-1} in goats.

Table 2. Kinetic Parameters of ACTP Ester[a] after Administration of a Single Intravenous Dose at 11.88 mg kg^{-1} in Goats ($n = 6$, Mean ± SE Values of both Male and Female)

parameter	value	parameter	value
C_B^0 (μg mL^{-1})	62.92 ± 6.26	f_c	0.23 ± 0.02
α (h^{-1})	0.81 ± 0.02	Cl_B (L kg^{-1} h^{-1})	0.04 ± 0.004
$t_{1/2(\alpha)}$ (h)	0.86 ± 0.03	K_{el} (h^{-1})	0.21 ± 0.02
β (h)$^{-1}$	0.05 ± 0.004	K_{12} (h^{-1})	0.46 ± 0.02
$t_{1/2(\beta)}$ (h)	14.83 ± 1.49	K_{21} (h^{-1})	0.19 ± 0.02
$V_{d,area}$ (L kg^{-1})	0.91 ± 0.19	K_{12}/K_{21}	2.45 ± 0.02
$V_{d,B}$ (L kg^{-1})	1.18 ± 0.26	AUC (μg h mL^{-1})	299.88 ± 25.5
$V_{d,ss}$ (L kg^{-1})	0.72 ± 0.14	Cl_R (L kg^{-1} h^{-1})	0.03 ± 0.004
$V_{d,c}$ (L kg^{-1})	0.19 ± 0.02	Cl_H (L kg^{-1} h^{-1})	0.009 ± 0.0006
T/B	3.55 ± 0.54		

[a] Test chemical. C_B^0, theoretical zero time blood herbicide concentration; α, rate constant related to slope of absorption curve; β, rate constant related to slope of elimination curve; $t_{1/2(\alpha)}$ and $t_{1/2(\beta)}$, half-lives of the herbicide in absorption and elimination phases, respectively; $V_{d,area}$, $V_{d,B}$, $V_{d,ss}$, and $V_{d,c}$, apparent volume of distribution of the herbicide on the total area under blood herbicide concentration versus time curve, distribution neglecting the absorption phase, distribution at steady state, and distribution in the central compartment, respectively; T/B, tissue/blood ratio; f_c, fraction of the amount of herbicide in the central compartment; Cl_B, total body clearance of the herbicide; K_{el}, first-order rate constant for herbicide elimination from central compartment; K_{12}, first-order rate constant for transfer of herbicide from central to peripheral compartment; K_{21}, first-order rate constant for transfer of herbicide from peripheral to central compartment; AUC, total area under the blood herbicide concentration versus time curve from 'O' to 'α' after administration of a single dose; Cl_R, renal clearance of the herbicide; Cl_H, hepatic clearance of the herbicide.

Recovery of ACTP Ester and Its Metabolites. *Urine.* Excretion of ACTP ester through urine following single intravenous administration was recorded at 24 h, and the maximum concentration was recorded at 48 h; thereafter, the ACTP ester excreted slowly until 96 h. Metabolites triclopyr acid and trichloropyridinol excreted through urine by 24 h reached a maximum in 24−48 h; thereafter, the concentration declined to below the detectable limit after 48−72 h (**Table 3**).

Table 3. Recovery of ACTP Ester with Its Metabolites, Triclopyr Acid and Trichloropyridinol, from Urine of Goats Following Administration of a Single Intravenous Dose at 11.88 mg kg^{-1}[a]

time (h)	ACTP ester (ppm)	triclopyr acid (ppm)	trichloro-pyridinol (ppm)
0–24	18.40 ± 3.56	13.63 ± 0.89	7.53 ± 0.67
24–48	76.35 ± 5.60	154.65 ± 10.40	133.02 ± 13.62
48–72	51.62 ± 6.21	56.53 ± 6.04	35.70 ± 2.42
72–96	17.52 ± 2.50	BDL	BDL

[a] Mean value of six replicates with SE. BDL, below detection limit.

Table 4. Recovery of ACTP Ester with Its Metabolites, Triclopyr Acid and Trichloropyridinol, from Feces of Goats Following Administration of a Single Intravenous Dose at 11.88 mg kg^{-1}[a]

time (h)	ACTP ester (ppm)	triclopyr acid (ppm)	trichloro-pyridinol (ppm)
0–24	23.53 ± 2.06	12.49 ± 1.11	1.12 ± 0.01
24–48	49.46 ± 4.50	36.77 ± 5.23	1.43 ± 0.03
48–72	16.80 ± 3.72	9.42 ± 0.19	0.02 ± 0.001
72–96	BDL	BDL	BDL

[a] Mean value of six replicates with SE. BDL, below detection limit.

Table 5. Blood Level of ACTP Ester after Repeated Oral Administration at 79.22 mg kg^{-1} for 7 Consecutive Days in Goats (n = 6, Mean ± SE Values of both Male and Female)

time (h)	ACTP ester (ppm)	time (h)	ACTP ester (ppm)
4[a]	8.65 ± 1.43	96[b]	3.72 ± 0.22
24[b]	6.50 ± 1.15	120[b]	4.81 ± 0.30
48[b]	4.19 ± 0.38	144[b]	4.60 ± 0.10
72[b]	5.51 ± 0.64		

[a] After oral administration. [b] Before successive oral administrations.

Feces. Excretion of ACTP ester and its metabolites, triclopyr acid and trichloropyridinol, through feces was recorded at 24 h, and maximum concentration was estimated at 48 h. The excretion was completed by 72 h (**Table 4**).

Nontoxic Repeated Oral Dose Study. Recovery of ACTP Ester and Its Metabolites. *Blood.* The concentration of ACTP ester in blood of goats was increased and decreased alternately on different days following repeated nontoxic oral dose administration at 79.22 mg kg^{-1} for a period of 7 consecutive days (**Table 5**).

Urine. Maximum concentration of ACTP ester was recovered from urine at 144−168 h, whereas maximum concentrations of metabolites were recovered from urine at 48−72 h after nontoxic repeated oral dose administration (**Table 6**). Stoichiometrically, recovery of parent compound was 2859.22 mg and the percentage of recovery against the administered dose was 51.56 (**Table 9**).

Feces. Maximum concentration of ACTP ester was recovered from feces at 48−72 h, whereas maximum concentrations of metabolites were recovered at 120−144 h after nontoxic repeated dose administrations (**Table 6**). Total quantity of ACTP ester and its metabolites in terms of parent compound was 247.02 mg, and the percentage of recovery against the administered dose was 4.45 (**Table 9**).

Gastrointestinal Tract Contents. Maximum concentrations of ACTP ester and trichloropyridinol were detected in the small intestine, whereas maximum concentration of triclopyr acid was recovered from the large intestine content (**Table 7**), after repeated nontoxic dose oral administration. Total quantity of ACTP ester and its metabolites in terms of parent compound was 101.16 mg, and the recovery percentage of ACTP ester against the administered dose was 1.82 (**Table 9**).

Tissue. The mean residual concentrations of ACTP ester and its metabolites recovered from different tissues following repeated oral administration are presented in **Table 8**. The parent compound as well as its metabolites could be recovered from all of the organs. Maximum concentration of ACTP ester could be recovered from skin followed by adrenal gland and abomasum in sequence. Total amount of recovered ACTP ester and its metabolites in terms of parent compound was 679.95, and total recovery percentage of ACTP ester was 12.26 (**Table 9**). Total recovery percentage of ACTP ester and its metabolites in terms of parent compound from different substrates was 70.09. Maximum percentage of recovery was recorded from urine, followed by tissues, feces, and gastrointestinal tract contents after repeated nontoxic dose oral administrations of ACTP ester (**Table 9**).

Metabolism in Goat. Only two major metabolites, namely, triclopyr acid and 3,5,6-trichloro-2 pyridinol, were quantified from urine, feces, tissues, and gastrointestinal content after intravenous (urine and feces only) and repeated oral administrations of ACTP ester. The concentration of metabolites was highest in urine followed by tissue, feces, and gastrointestinal contents after repeated nontoxic oral dosing.

Microsomal Study. Mean cytochrome P_{450} contents of the liver microsomal pellet of groups 1, 2, and 3 were 1.11 ± 0.06, 1.29 ± 0.13, and 2.27 ± 0.06 nmol mg^{-1} of microsomal protein, respectively (**Table 10**). Phenobarbitone-treated goats showed significant ($P < 0.01$) increase of cytochrome P_{450} content of liver microsomal pellet, whereas ACTP ester treated goats did not vary in cytochrome P_{450} content from the control value.

Table 6. Recovery of ACTP Ester with Its Metabolites, Triclopyr Acid and Trichloropyridinol, from Feces and Urine of Goats Following Repeated Oral Dose Administration for 7 Consecutive Days at 79.22 mg kg^{-1}[a]

	feces			urine		
time (h)	ACTP ester (ppm)	triclopyr acid (ppm)	trichloro-pyridinol (ppm)	ACTP ester (ppm)	triclopyr acid (ppm)	trichloro-pyridinol (ppm)
0–24	103.59 ± 18.65	47.82 ± 0.25	9.90 ±0.09	68.88 ± 19.21	413.82 ± 60.08	304.19 ± 50.00
24–48	194.77 ± 57.44	33.04 ± 0.23	13.05 ± 0. 15	67.74 ± 4.77	1209.4 ± 100.02	397.48 ± 45.00
48–72	381.11 ± 45.96	48.35 ± 0.19	27.32 ± 0.17	174.82 ± 17.96	3122.98 ± 200.04	754.03 ± 80.20
72–96	268.51 ± 41.99	51.77 ± 0.35	11.58 ± 0.13	118.86 ± 29.38	1136.48 ± 150.07	277.88 ± 28.80
96–120	202.06 ± 48.26	41.91 ± 0.32	15.45 ± 0.15	122.48 ± 20.92	1065.07 ± 135.28	282.28 ± 29.73
120–144	87.95 ± 9.41	53.73 ± 0.34	31.23 ± 0.28	156.16 ± 19.24	913.07 ± 85.15	240.23 ± 23.75
144–168	105.37 ± 28.96	65.42 ± 0.19	19.65 ± 0.17	345.35 ± 35.64	1458.05 ± 165.85	431.97 ± 55.08

[a] Mean value of six replicates with SE.

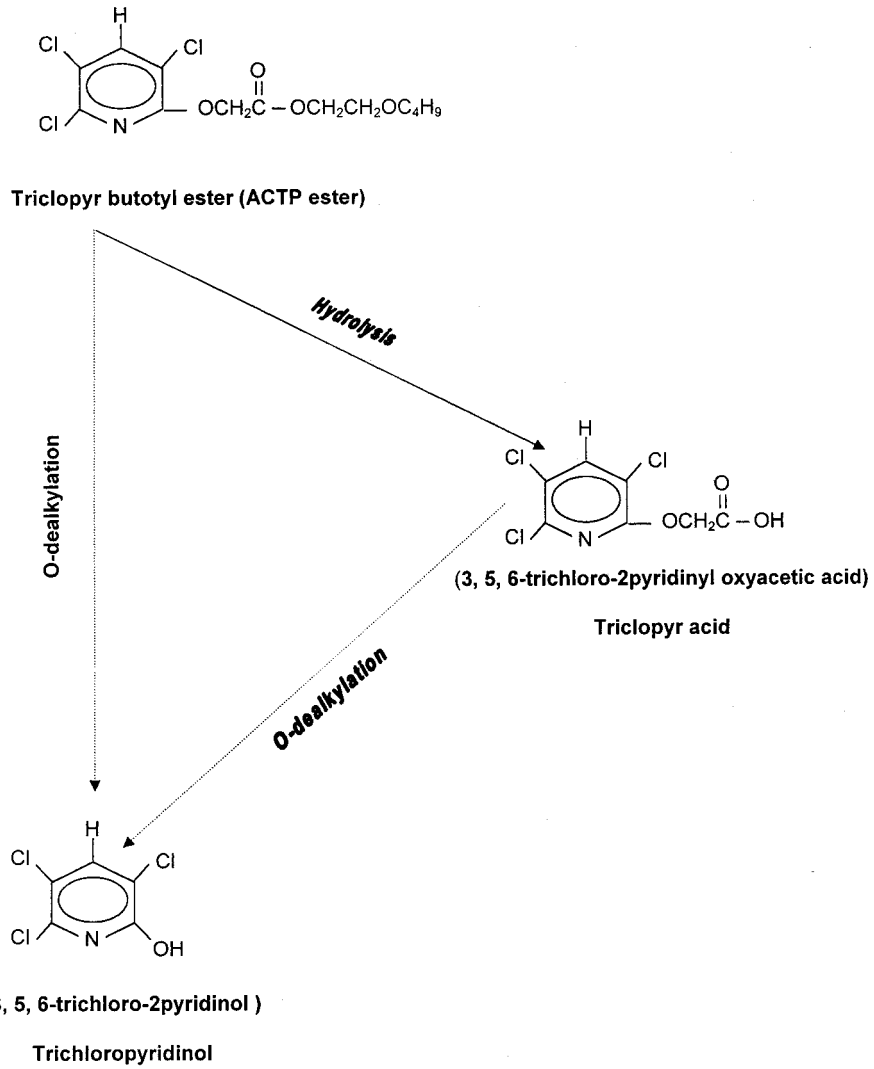


Figure 3. Proposed metabolic pathway of ACTP ester.

Table 7. Recovery of ACTP Ester and Its Metabolites, Triclopyr Acid and Trichloropyridinol, from Gastrointestinal Contents of Goats Following Repeated Oral Dose Administration for 7 Consecutive Days at 79.22 mg kg^{-1}[a]

substrate, content from	ACTP ester (ppm)	triclopyr acid (ppm)	trichloro-pyridinol (ppm)
rumen	16.86 ± 1.11	0.83 ± 0.19	4.27 ± 0.59
small intestine	119.98 ± 13.16	28.58 ± 3.25	52.36 ± 4.45
large intestine	28.55 ± 4.89	30.51 ± 4.15	25.11 ± 2.89

[a] Mean value of six replicates with SE.

DISCUSSION

Toxicokinetic parameters such as distribution rate constant (α) and distribution half-life ($t_{1/2(\alpha)}$) associated with the rate constant for transfer of herbicide from central to peripheral compartments (k_{12}) after intravenous administration indicate a slow rate of distribution of ACTP ester. Concurrently, greater elimination half-life ($t_{1/2(\beta)}$) coupled with poor body clearance (Cl_B) and renal clearance (Cl_R) values indicates longer persistence of the compound in blood as well as slow excretion of the parent compound and its metabolites in urine until 96 and 72 h, respectively. Despite the poor hepatic clearance (Cl_H) value compared to renal clearance (Cl_R) value, both the parent and its metabolite could be recovered from the feces of goat until 72 h after intravenous administration.

Table 8. Recovery of ACTP Ester and Its Metabolites, Triclopyr Acid and Trichloropyridinol, from Tissues of Goats Following Repeated Oral Dose Administration for 7 Consecutive Days at 79.22 mg kg^{-1}[a]

tissue	ACTP ester (ppm)	triclopyr acid (ppm)	trichloro-pyridinol (ppm)
liver	48.08 ± 5.15	58.27 ± 0.99	59.10 ± 3.34
lung	55.05 ± 5.85	48.96 ± 0.43	54.93 ± 6.75
heart	42.75 ± 7.56	37.64 ± 3.21	24.88 ± 0.17
kidney	46.18 ± 4.90	87.81 ± 8.21	122.07 ± 6.85
brain	46.48 ± 2.73	18.86 ± 0.17	14.81 ± 0.12
fat	28.73 ± 3.51	74.78 ± 6.74	63.20 ± 6.41
spleen	12.48 ± 1.43	33.92 ± 0.17	17.92 ± 0.15
bile	18.09 ± 4.03	147.81± 10.12	96.53 ± 8.10
adrenal gland	87.51 ± 5.07	735.84 ± 10.09	572.32 ± 10.01
ovary	78.66 ± 3.71	719.29 ± 10.06	666.66 ± 12.04
uterus	15.32 ± 3.82	67.22 ± 6.18	113.19 ± 8.05
muscle	30.76 ± 5.99	0.50 ± 0.16	3.02 ± 0.28
rumen	68.23 ± 4.74	27.48 ± 1.24	36.96 ± 3.38
reticulum	42.82 ± 6.24	44.94 ± 5.10	49.39 ± 4.15
omasum	53.36 ± 6.58	35.12 ± 2.16	37.68 ± 3.15
abomasum	84.23 ± 9.61	58.39 ± 3.18	47.09 ± 5.21
large intestine	39.39 ± 4.47	11.34 ± 0.92	45.00 ± 5.69
small intestine	63.32 ± 6.68	48.73 ± 3.05	33.31 ± 3.49
skin	91.79 ± 5.63	26.61 ± 1.32	44.88 ± 2.04
bone	10.36 ± 1.01	13.84 ± 2.01	11.06 ± 1.47
testis	26.00 ± 2.94	9.50 ± 1.03	7.96 ± 0.97
blood	2.71 ± 0.14	2.22 ± 0.13	BDL

[a] Mean value of six replicates with SE. BDL, below detection limit.

Table 9. Total Recovery Percentage of ACTP Ester from Goats Following Repeated Oral Dose Administration for 7 Consecutive Days at 79.22 mg kg^{-1}

substrate	% recovered
urine	51.56 (2859.22)[a]
feces	4.45 (247.02)[a]
gastrointestinal contents	1.82 (101.16)[a]
tissues	12.26 (679.95)[a]
total	70.09

[a] Figure in parentheses indicates total quantity (mg) recovered.

Table 10. Effect of ACTP Ester on Cytochrome P_{450} Contents of Liver Microsomal Pellet of Goats Following Repeated Oral Administration at 79.22 mg kg^{-1} for 7 Days[a]

	animal					
group	1	2	3	4	mean ± SE	*F* value[b]
1 (control)[c]	1.14	1.05	0.99	1.24	1.11 ± 0.06	
2 (ACTP ester)[c]	1.58	1.04	1.31	1.26	1.29 ± 0.13	2.424[NS]
3 (phenobarbitone)[d]	2.40	2.25	2.15	2.29	2.27 ± 0.06	241.33**

[a] Cytochrome P_{450} results reported as nmol mg^{-1} of microsomal protein. [b] NS, nonsignificant between groups 1 and 2. **, $P < 0.01$ between groups 1 and 3. [c] Goats slaughtered after 7 days. [d] Goats slaughtered after 5 days.

The recovery percentage of ACTP ester after nontoxic oral dosing for 7 days was 70.09, of which 4.45% was recovered from feces, indicating that a major portion of the orally administered ACTP ester was absorbed from the gastrointestinal tract. The quantity of ACTP ester recovered from the goat feces includes the absorbed portion excreting through bile and a nonabsorbed portion. The apparent volume of distribution ($V_{d,area}$) value of the toxicokinetic study was found to be normal, indicating normal distribution of herbicide to all parts of the body and, in accordance with the observation, all of the goat tissues after repeated dosing retained ACTP ester and its metabolitess; the recovery percentage was 12.26. Skin retained the maximum residue followed by adrenal gland; skin contains a sufficient amount of fat, and therefore lipophilicity might be one of the contributing factors for retaining a good amount of residue. ACTP ester recovered from goat urine was 2859.22 mg, of which 171.72 mg was excreted as parent compound, whereas the remaining 2687.50 mg of ACTP ester was excreted as metabolites. More than 50% of the administered ester was recovered from urine. The results of the intravenous toxicokinetic study also indicated that urine is the major pathway of excretion of the compound. ACTP ester did not alter the cytochrome P_{450} content of liver tissue. Microsomal and nonmicrosomal esterase activities were not assayed in this study, but, on the basis of the identification of two metabolites, namely, 3,5,6-trichloro-2-pyridinyloxyacetic acid (triclopyr acid) and 3,5,6-trichloro-2pyridinol, a tentative metabolic pathway (**Figure 3**) was proposed, in which both hydrolysis and O-dealkylation can proceed side by side. Therefore, the possible involvement of microsomal esterase in the metabolism of ACTP ester cannot be ruled out.

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LITERATURE CITED

(1) Tomlin, C. D. S., Ed. *The Pesticide Manual*, 11th ed.; British Crop Protection Council: Surrey, U.K., 1997.

(2) Eckerlin, R. H.; Ebel, J. G., Jr.; Maylin, G. A.; Muscato, T. V.; Gutenmann, W. H.; Bache, C. A.; Lisk, D. J. Excretion of Triclopyr Herbicide in the Bovine. *Bull. Environ Contam. Toxicol.* **1987**, *39* (3), 443−447.

(3) Juliet, S.; Mandal, T. K.; Mal, B.; Chowdhury, A.; Bhattacharyya, A.; Chakraborty, A. K. Metabolic Study of Isoproturon in Goats Following a Single Oral Administration: Toxicokinetics and Recovery. *J. Agric. Food Chem.* **1998**, *46*, 178−183.

(4) Carmichael, N. G.; Nolan, R. J.; Perkins, J. M.; Davies, R.; Warrington, S. J. Oral and Dermal Pharmacokinetics of Triclopyr in Human Volunteers. *Toxicology* **1989**, *8*, 431−437.

(5) Baggot, J. D. *Principles of Drug Disposition in Domestic Animals. The Basis of Veterinary Clinical Pharmacology*; Saunders: Philadelphia, PA, 1977.

(6) Davis, N.; Davis, L. E.; Powers, T. E. Comparative Body Compositions of the Dog and Goat. *Am. J. Vet. Res.* **1975**, *36*, 309−311.

(7) Lowry, O. H.; Rosenbrough, N. J.; Farr, A. L.; Randal, R. J. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **1951**, *243*, 1331−1332.

(8) Omura, T.; Sato, R. The Carbon Monoxide Binding Pigment of Liver Microsome. 1. Evidence for Its Haemoprotein Nature. *J. Biol. Chem.* **1964**, *239*, 2370−2378.

(9) Snedecor, G. W.; Cochran, W. G. *Statistical Methods*, 6th ed; Iowa State University Press: Ames, IA, 1968.

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