Toxico-Kinetics, Recovery, and Metabolism of Napropamide in Goats Following a Single High-Dose Oral Administration

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Toxicokinetic behavior, recovery and metabolism of napropamide (a pre-emergent herbicide) and its effect on Cytochrome P_{450} of liver microsomal pellet were studied following a single high-dose oral administration of 2.5 g kg^{-1} and continuous (7 days) oral administration of 500 mg kg^{-1} in black Bengal goat. Napropamide was detected in blood at 15 min and the maximum quantity was recovered at 3 h after administration. The absorption rate constant (*Ka*) value was low indicating poor absorption from the gastrointestinal tract. High elimination half-life ($t_{1/2}$ β) and low body clearance (Cl_B) values coupled with higher transfer of compound from tissue to central compartment (K_{21}) suggest that napropamide persisted in the blood for a long time, i.e., after 72 h of oral administration. The recovery percentage of napropamide, including metabolites, from goats varied from 75.94 to 80.08 and excretion of the parent compound through feces varied from 18.86 to 21.59%, indicating that a major portion of the orally administered napropamide was absorbed from the gastrointestinal tract of goat. Napropamide significantly increased the Cytochrome P_{450} content of liver microsomal pellet. The recovery of metabolites from feces, urine, and tissues ranged from 4.2−6.2, 40.81−49.42, and 2.7−11.6%, respectively, during a 4−7 day period. The material balance of napropamide (including metabolites) following a single high-dose oral administration at 2.5 g kg^{-1} during 4−7 days after dosing was found to be in the range of 75−80%.

Keywords: *Napropamide; persistence metabolism; effect on Cytochrome P_{450}; recovery; goat; herbicide; pesticide*

INTRODUCTION

Napropamide [N,N-diethyl-2-(1-naphthalenyloxy) propanamide] is widely used as a pre-emergent herbicide for the control of many annual grasses and broad leaf weeds. The herbicidal property of napropamide was first reported by Chan et al. (*1*). Enhanced biodegradation of the herbicide in soil was observed by Walker et al. (*2*−*4*). The photodecomposition of formulated napropamide in soil has been demonstrated; its aqueous photolysis produced transformation products involving molecular rearrangement and coupling (*5*). However, no information on the disposition kinetics and metabolism of napropamide is available in the literature, especially in the animal system. The objective of the present study was to evaluate the toxico-kinetic behavior, distribution, retention, and metabolism of napropamide in different tissues of goat following oral administration of a single high dose. The results obtained from this study will be of value in assessing the possible health hazards posed by the use of napropamide.

EXPERIMENTAL PROCEDURES

Chemicals. Napropamide (technical grade, purity 97%) and its metabolites α-naphthoxy propionic acid (purity 96.5%), 1,5-dihydroxy naphthalene (purity 97.5%), and *N*-ethyl napropamide (purity 97.5%), the three most probable metabolites, were supplied by M/s. Gharda Chemicals Ltd., Mumbai, India. All these compounds were further purified and were authenticated by HPLC and spectroscopic (UV, IR, MS, and NMR) analyses. All other chemicals and solvents used in this study were of analytical grade (E. Merck, India).

Animal Treatment. Clinically healthy adult black Bengal male (nulliparous) and female goats weighing between 9.5 and 12 kg were selected. The goats were acclimated individually in stainless steel metabolism cages and provided with artificial fluorescent lighting, controlled temperature (22 ± 3 °C), water, and standard feed (*6*). Each animal was fasted overnight before treatment.

For ascertaining the minimum oral toxic dose level, four different doses of napropamide suspended in carboxymethyl cellulose (1% w/v) were administered to four groups of goats separately, each group made up of one male and one female. Likewise, for ascertaining the maximum oral nontoxic dose level after administering a single dose for 7 consecutive days, three different doses of napropamide suspended in carboxymethyl cellulose (1% w/v) were administered to three groups of goats separately, each consisting of one male and one female goat.

For the metabolic study of napropamide 14 male and 14 female goats were utilized. Of these, two males and two females were kept as controls and received the vehicle (carboxymethyl cellulose) only. The remaining male and female goats were divided into four groups, each made up of three male and three female animals, which were orally treated with napropamide suspension in carboxymethyl cellulose. A group of treated animals was sacrificed on each of days 4, 5, 6, and 7 days.

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10.1021/jf991387w CCC: $20.00
Published on Web 03/07/2001

For microsomal study six male and six female goats were considered and divided into three equal groups, each having 2 male and 2 female goats.

Fixation Of Single Minimum Oral Toxic Dose. For ascertaining a single maximum oral dose level of napropamide which would exhibit minimum toxic symptoms in goat without causing mortality during the observation period of 7 days, Napropamide at four dose levels (1.0, 2.0, 2.5, and 3.0 g kg^{-1}) suspended in carboxymethyl cellulose solution (1% w/v) was orally administered once to each of the animals of different groups, respectively. They were then kept under observation. It has been observed that oral administration of napropamide at 2.5 g kg^{-1} produced the signs of toxicity, without causing mortality during the observation period. The toxic symptoms observed include drowsiness within 20 min, grinding of teeth, increased rate and depth of respiration, and moderate bloat which persisted until 8 h after dosing. Goats treated orally with napropamide at 3 g kg^{-1} produced the above symptoms with greater intensity. Accordingly, the single maximum oral dose level of napropamide has been fixed of 2.5 g kg^{-1} in the present study.

Fixation Of Maximum Non-Toxic Oral Dose. The objective was to find a dose level of napropamide which on single administration for 7 consecutive days would not produce any toxic symptoms. Napropamide at three dose levels (200 mg, 500 mg, and 1 g) suspended in carboxymethyl cellulose (1% w/v) was administered orally once daily to each animal of different groups, respectively, for 7 consecutive days. The goats treated with napropamide at 1 g kg^{-1} exhibited the toxic symptoms such as piloerection and bloat after 6 days of administration, whereas dose levels of 200 and 500 mg kg^{-1} did not exhibit any sign of illness within the observation period. Accordingly, napropamide at 500 mg kg^{-1} was considered the maximum oral nontoxic dose.

Kinetics. For kinetic study, blood samples were collected from the right jugular vein of each experimental goat. Samples were collected in hepa-rinsed tubes at different time intervals (0.25, 0.50, 1.5, 2.0, 2.5, 3, 4, 6, 8, 10, 12, 24, 48, 72, 96, 120, 144, and 168 h) during napropamide administration. The concentration of napropamide was estimated by HPLC. The values of kinetic parameters such as Ka, $t_{1/2}$ (Ka), β, $t_{1/2}$ (β), Vd area, AUC, Kel, Cl_B, Cl_H, Cl_R, $T{\sim}B$, K_{21}, and K_{12} were determined from the semilogarithmic plots of blood-level time profile data in goats using standard formulas (*7*).

Collection of Feces and Urine. Feces and urine of individual goats were collected at 24, 48, 72, 96, 120, 148, and 168 h after administration. The excretions were measured or weighed and stored at −20 °C prior to extraction.

Collection of Tissues, Bile, and Gastrointestinal Contents. The animals of the first, second, third, and fourth groups were killed on 4, 5, 6, and 7 days after dosing, respectively. Samples of liver, kidney, lungs, brain, heart, spleen, adrenal gland, muscle, omental fat, ovary, uterus, testis, rumen, reticulum, omasum, abomasum, skin, bone, bile, and contents of rumen and intestines were taken, weighed, chopped, and stored at −20 °C prior to extraction.

Microsomal Study. Napropamide at 500 mg kg^{-1} and suspended in carboxymethyl cellulose (1% w/v) was administered orally once daily for 7 continuous days to each goat of group 2, but only carboxymethyl cellulose was administered orally in the aforesaid procedure to each goat of group 1 (control). Phenobarbitone sodium (Gardenal, R-P) 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 killed on day 8 of administration. Pieces of liver (Candate 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 [tri-hydrochloride (10 mM, pH 7.4) containing KCl (0.1 M), ethylenediamine tetra-acetic 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 105 000*g* for 1 h in a Micro-ultracentrifuge to yield a microsomal pellet. The microsomal pellet was suspended in buffer [potassium pyrophosphate (0.1 M, pH 7.4) containing ethylenediamine tetra-acetic acid (1 mM) and butylated hydroxytoluene (20 μM)], homogenized with four passes of a mechanically driven Teflon-glass homogenizer, and again recentrifuged at 105 000*g* for 1 h. The supernatant fraction was decanted and the microsomal pellet was resuspended in a volume of buffer [tris-hydrochloride (10 mM, pH 7.4), ethylenediamine tetra-acetic acid (1.0 mM), and glycerol (20%, v/v)] and Cytochrome P_{450} was immediately measured. Protein was measured by the method described by Lowery et al (*8*).

Extraction and Cleanup. Acetonitrile (1 mL) and ethyl acetate (40 mL) were added to the blood sample (1 mL). The mixture was shaken and allowed to settle. The supernatant was collected in a conical flask. Ethyl acetate (20 mL) was added to the blood precipitate and the above step was repeated twice. The combined supernatant was evaporated to dryness using a rotary vacuum evaporator at 40 °C. The dry precipitate in the flask was washed with 5−10 mL of acetonitrile, shaken well, and passed through anhydrous sodium sulfate (∼ 4 g). The filtrate was evaporated to dryness using a rotary vacuum evaporator at 40 °C and the residue was dissolved in 1 mL of acetonitrile and transferred to a volumetric flask for subsequent HPLC analysis.

The method of extraction from feces (2 g) is presented in Scheme 1. Extractions of intestinal and ruminal contents were done in the same way as described for feces extraction.

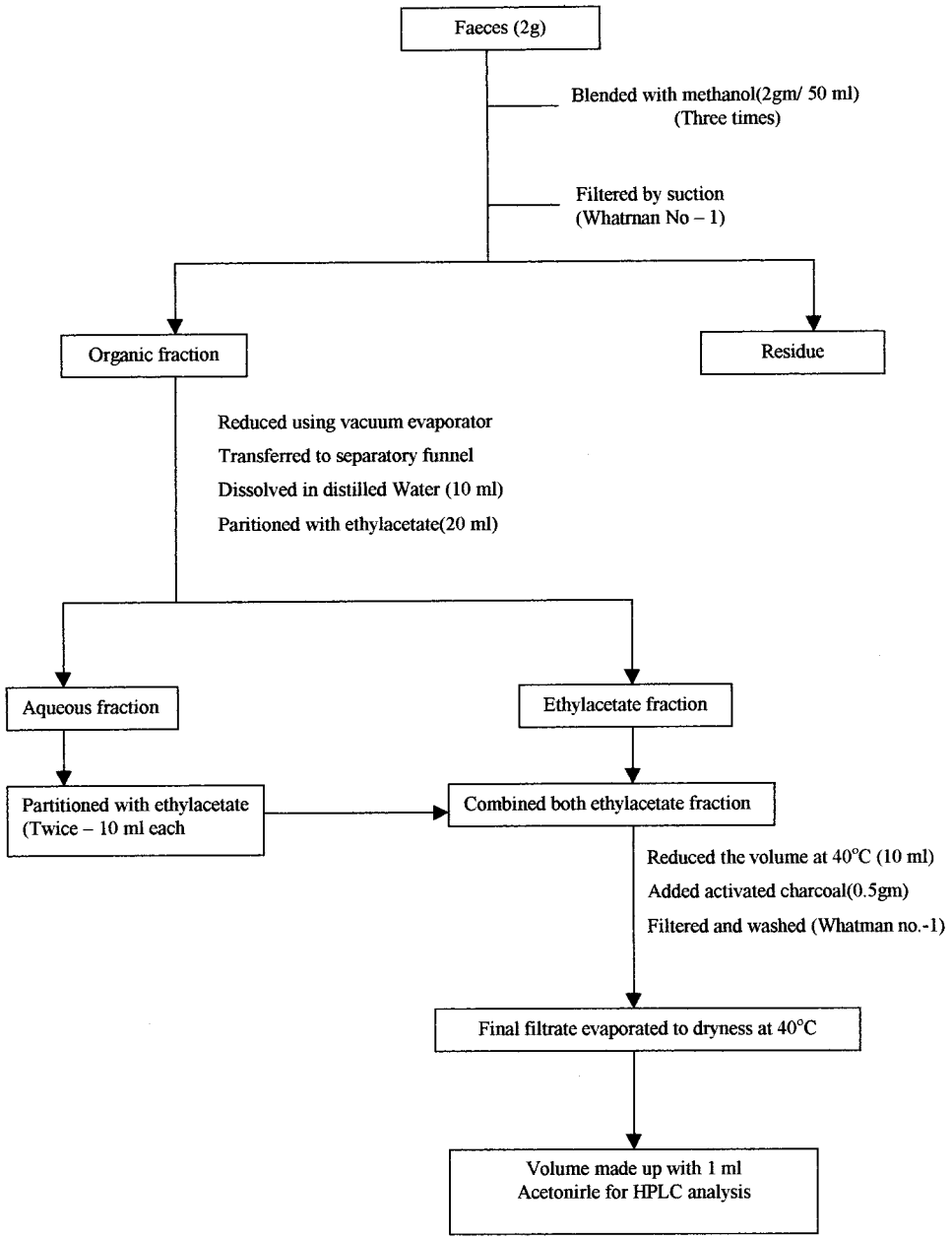
Urine. Distilled water (50 mL) was added to each 50 mL of urine sample and the mixture was extracted with diethyl ether (50 mL × 3) in a separatory funnel for 2−3 min. The combined diethyl ether fraction was passed through anhydrous sodium sulfate (4 g), taken into a 250-mL flask and evaporated to dryness using a rotary vacuum evaporator at 40 °C. The residue thus obtained was dissolved in 1 mL of acetonitrile for further analysis by HPLC.

Bile. To a sample of 2 mL of bile in a 50-mL stoppered test tube, 1 mL of acetonitrile and 20 mL of methanol were added. The mixture was shaken for 5 min and allowed to settle and the supernatant was collected in a conical flask. The residue was extracted with methanol (20 mL × 2). The combined supernatant was transferred to a clean dry 125-mL evaporating flask and evaporated to dryness using a rotary vacuum evaporator at 40 °C. The dried extract was dissolved in 5 mL of acetonitrile in the evaporating flask. The extract mixture was shaken and passed through 4 g of anhydrous sodium sulfate. The acetonitrile portion was reduced to a 1 mL by rotary vacuum evaporation at 40 °C and transferred to a volumetric flask for subsequent HPLC analysis.

Tissues. A small quantity of tissue (2 g) except fat, skin, and bone was first minced and then homogenized in a 50-mL homogenizing cup with 25 mL of acetonitrile and 0.5 g of anhydrous sodium sulfate by a homogenizer (4000 rpm) for 4 min. The extracts were filtered through 0.5 g of anhydrous sodium sulfate, and the remnants were rehomogenized and reextracted twice with 25 mL and 12 mL of acetonitrile. The acetonitrile extract was clarified by centrifugation. The combined acetonitrile extracts were concentrated to 20 mL using rotary vacuum evaporation at 40 °C. The extract was transferred to a clean dry separatory funnel (125 mL), 100 mL of *n*-hexane was added to it, and the mixture was shaken vigorously for 5 min. It was then allowed to settle for 3−5 min until the hexane and acetonitrile phases were separated; this partition step was repeated twice. Finally, the acetonitrile phase was evaporated to dryness at 40 °C and the residue was dissolved in 1 mL of acetonitrile for subsequent HPLC analysis.

Fat tissue (1 g) was minced and homogenized with 15 mL of *n*-hexane and 0.5 g of sodium sulfate at 4000 rpm for 4 min and filtered through 0.4 g of anhydrous sodium sulfate. The residue was re-extracted twice with 10 mL of *n*-hexane. The combined filtrates were transferred to a clean dry evaporating flask and the filtrate was concentrated to 5 mL in volume.

Scheme 1. Extraction and Cleanup of Caprine Feces



The filtrate was again transferred to a separatory funnel, 10 mL of acetonitrile was added, and the mixture was shaken vigorously for 5 min. After separation of the two phases, the lower acetonitrile phase was transferred to another separatory funnel and 5 mL of *n*-hexane was added to it and shaken for 5 min. The lower acetonitrile phase was transferred to a separatory funnel. To the upper hexane phase we added 10 mL of acetonitrile and shook the mixture for 5 min. The hexane phase was discarded and the acetonitrile phase was taken in a clean dry evaporating flask and evaporated to dryness in a rotary vacuum evaporater at 40 °C. The residue was dissolved in 1 mL of acetonitrile for subsequent HPLC analysis.

Skin was shaved and chopped up. A 2-g aliquot was transferred to a 50-mL conical flask with 20 mL of methanol and kept under refrigeration for 24 h. The sample was then homogenized for 4 min, filtered through 4 g of sodium sulfate, and collected in a conical flask. The residue was extracted twice with 20 mL of methanol. The combined filtrate was concentrated to 10 mL volume. It was transferred to a separatory funnel, 25 mL of hexane was added, and the mixture was shaken for 5 min. The lower phase was transferred to a separatory funnel and the partition was repeated twice. The lower phase was evaporated to dryness, and the residue was dissolved in 1 mL of acetonitrile for HPLC analysis.

Bone (20 g) was crushed and ground for 5 min. The sample was transferred into a 100-mL conical flask with 50 mL of ethyl acetate and kept under refrigeration for 24 h. The sample was then filtered through 4 g of sodium sulfate. The residue was washed twice with 20 mL of ethyl acetate and filtered through 4 g of sodium sulfate. The combined filtrate was transferred to an evaporating flask and the volume was reduced to 10 mL at 40 °C. Acetonitrile (20 mL) was added to the extract and the solution was transferred to a separatory funnel with 25 mL of hexane. The funnel was capped tightly and shaken for 5 min. The lower phase was transferred to a separatory funnel. This step was repeated twice. The lower phase was evaporated to dryness and the residue was dissolved in 1 mL of acetonitrile for subsequent HPLC analysis.

Quantification. A Hewlett-Packard (model 1050) liquid chromatograph coupled with a variable wavelength UV−Vis detector and a 3392A integrator was used for the analysis of napropamide and it metabolites. Following are the operational parameters that were used: mobile phase, acetonitrile/water

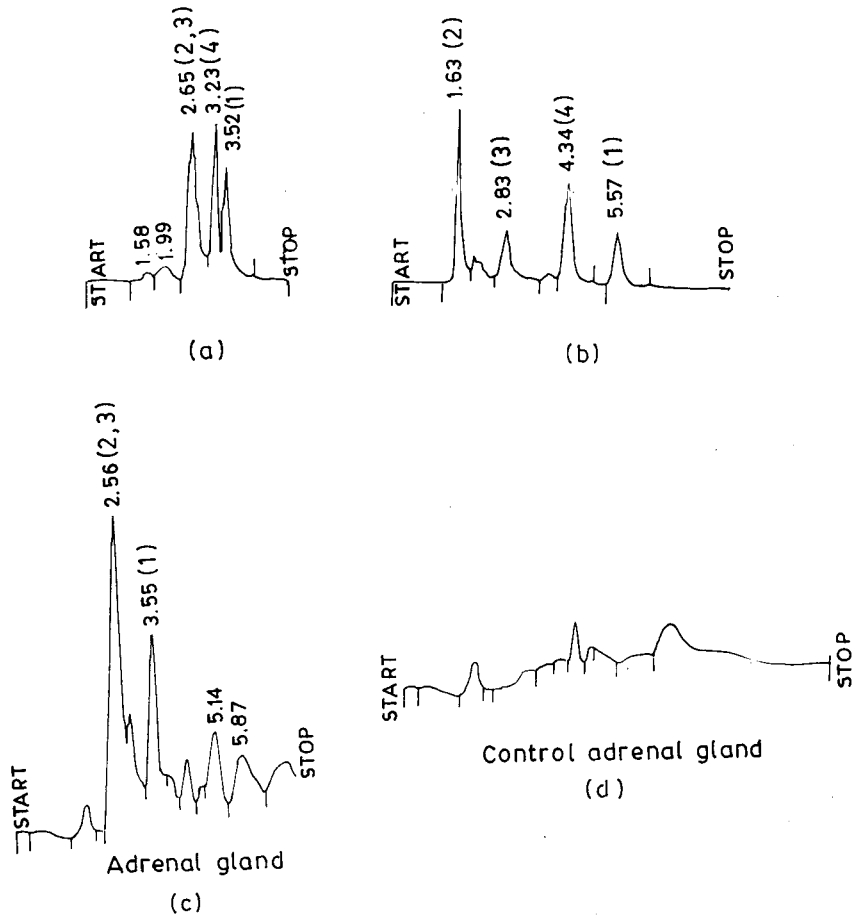


Figure 1. HPLC chromatograms of Napropamide and its metabolites (a) analytical standards in acetonitrile/water (9:1 v/v); (b) analytical standards in acetonitrile/water (7:3 v/v); (c) cleaned-up extract from the tissues (adrenal gland); (d) control extract from the tissues (adrenal gland).

(9:1 and 7:3 v/v); flow rate, 1 mL min^{-1}; column, Shandon Hypersil HPLC column 250 × 4.6 mm; ODS, 5 μ (RPC $_{18}$); injection, Hamilton microliter syringe (25 μL); detector wavelength, 280 nm; total run, 15 min.

Standard solutions (10 μg/mL) of the analytical grade napropamide and its metabolites in acetonitrile were injected into the HPLC maintained with the above operating conditions. The retention times (*RT*) observed for napropamide and its metabolites in two solvent systems are shown in Figure 1. The cleaned-up extracts of samples were injected and compared with the RT of standard solution. The amount of napropamide and its metabolites in respective samples were obtained by comparison with external standard.

Measurement of Cytochrome P_{450} Contents. Cytochrome P_{450} contents of microsomal pellets were estimated according to the methods of Omura and Sato (*9*). 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 Beckman DU 6 spectrophotometer. 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} cm^{1-} for $A_{409-490}$ nm.

Statistical Analysis of Data. Statistical analyses of data were conducted by using standard formulas (*10*).

RESULTS

Recovery. The recoveries of napropamide and its metabolites were estimated by fortifying different substrates with known quantities to give final concentrations of 0.10, 0.25, 0.50, and 1.0 ppm for blood and urine and 0.50, 1.0, 2.5, and 5.0 ppm for feces and other tissues. The linearity for all the compounds was checked by calibration curve. The recoveries of napropamide and it metabolites from blood, urine, feces, and other tissues varied from 80 to 92%. The limit of detection of napropamide and its metabolites was found to be 0.01 ppm.

Napropamide in Blood. The initial concentration of napropamide in blood at 15 min. was found to be 1.30 ± 0.05 μg mL^{-1}. The maximum concentration was detected at 3 h (32.5 ± 4.15 μg mL^{-1}) and thereafter the concentration declined until 72 h. Napropamide could not be detected in blood samples at 96 h (Figure 2). The kinetic behavior followed a two-compartment open model. The *Vd* (area), β, $t_{1/2}$ (β), *Ka*, and $\tilde{T}\sim B$ of napropamide were 0.20 ± 0.03 (L kg^{-1}), 0.08 ± 0.005 (h^{-1}), 9.12 ± 1.50 (h), 0.48 ± 0.16 (h^{-1}), and 0.89 ± 0.17 respectively, and Cl_B, Cl_R, Cl_H, K_{21}, and K_{12} values were, respectively, 0.02 ± 0.001 (L $kg^{-1}h^{-1}$), 0.005 ± 0.0001 (L $kg^{-1}h^{-1}$), 0.26 ± 0.09 (h^{-1}), and 0.16 ± 0.02 (h^{-1}) (Table 1).

Recovery of Napropamide and its Metabolites. *Feces.* Excretion of napropamide through feces was recorded at 24 h, and the maximum quantity was recorded in the 24−48 h sample. The quantity of napropamide excreted then decreased slowly. Recovery percentages of napropamide excreted through feces against total quantity administered on days 4, 5, 6, and

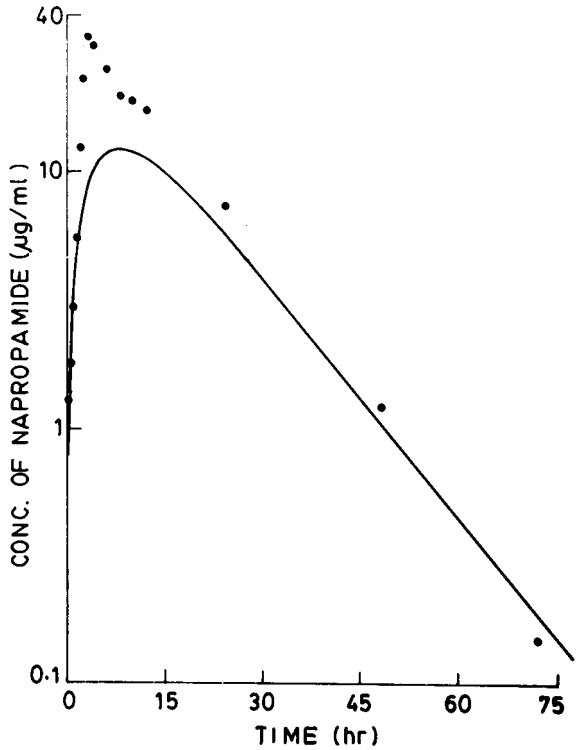


Figure 2. Semilogarithmic plot of blood napropamide concentration against time with computerized best-fit line after oral administration of single dose at 2.5 g kg^{-1} in goats.

Table 1. Kinetic Parameters for Napropamide[a] after a Single High-Dose Oral Administration to Goats at 2.5 g kg^{-1} (n = 6; Mean SE Values of both Male and Female)

parameter	value
Ka (h^{-1})	0.48 ± 0.16
$t_{1/2}$ Ka (h)	1.44 ± 0.68
β (h^{-1})	0.08 ± 0.005
$t_{1/2}$ (β) (h)	9.12 ± 1.50
Vd area (L kg^{-1})	0.20 ± 0.03
AUC (μg h mL^{-1})	644.21 ± 54.33
Cl_B (L kg^{-1} h^{-1})	0.02 ± 0.001
Cl_R (L kg^{-1} h^{-1})	0.005 ± 0.0001
Cl_H (L kg^{-1} h^{-1})	0.015 ± 0.003
K_{21} (h^{-1})	0.26 ± 0.09
K_{12} (h^{-1})	0.16 ± 0.02
$T \sim B$	0.89 ± 0.17
Kel	0.14 ± 0.01

[a] Test chemical.

7 after dosing were 18.86, 21.59, 20.18, and 19.85%, respectively. Metabolites were excreted through feces by 24 h, reached a maximum in the 24−48 h feces sample and thereafter the concentration declined to below the detectable limit after the 120−144 h sample. Recovery percentages of napropamide metabolites excreted through feces were 4.20, 4.26, 5.82, and 6.23 on days 4, 5, 6, and 7 after dosing, respectively (Table 2).

Urine. The excretion pattern of napropamide and its metabolites through urine resembled that of feces. The rate of excretion was initially slow, attained a maximum in the 24−48 h urine sample and thereafter declined gradually and completed in the 72−96 h urine in all the goat groups. Percentages of napropamide recovered from urine of goats on days 4, 5, 6, and 7 after dosing were 0.63, 0.96, 0.89, and 0.56%, respectively. On the other hand, metabolites were present in urine in higher concentrations when compared to those of the parent compound. The maximum quantity of metabolites was found to be present in the 24−48 h urine sample in all the groups of goats and elimination was completed in the 120−144 h sample. Percentages of metabolite recovered from urine of goats on days 4, 5, 6, and 7 after dosing were 40.81, 43.03, 45.40, and 49.42, respectively. (Table 3).

Tissues. The mean residual content of napropamide and its metabolites recovered from different tissues of goats are presented in (Table 4). The maximum quantities of parent compound and its metabolites were recovered from goats on day 4 after administration. The percentages of recovery of parent compound were 1.93, 1.87, 0.47, and 0.14 and those of metabolites were 11.39, 8.37, 3.18, and 2.68 from tissue of goats on days 4, 5, 6, and 7 after dosing, respectively. Total recovery percentages from different tissues for napropamide and its metabolites from groups of goats on days 4, 5, 6, and 7 after dosing were 78.02, 80.08, 75.94, and 78.88 respectively (Table 5).

Metabolism in Goat. Three major metabolites, namely α-naphthoxy propionic acid (**2**), 1,5-dihydroxy naphthalene (**3**), and *N*-ethyl napropamide (**4**), were identified from urine, feces, and tissues. The concentration of metabolites was highest in urine, followed by the concentrations in tissues and feces.

Microsomal Study. Mean Cytochrome P_{450} contents of the liver microsomal pellet of groups 1, 2, and 3 were 1.07 ± 0.03, 2.92 ± 0.02, and 2.22 ± 0.04 n mole mg^{-1} of microsomal protein respectively (Table 6). Both napropamide- and phenobarbitone-treated goats showed significant ($P < 0.01$) increase of Cytochrome P_{450} contents of liver microsomal pellet.

DISCUSSION

Napropamide was detected in the blood at 15 min and the maximum quantity was recovered at 3 h following oral administration. This indicates that the rate of absorption of napropamide from the gastrointestinal tract of goat was poor and supplemented the poor Ka value. High elimination half-life ($t_{1/2}\beta$) and low body clearance (Cl_B) values coupled with high K_{21} value (transfer of parent compound from periphery to central compartment) suggest that napropamide persisted in the blood for a long time and accordingly the compound could be detected in blood until 72 h after administration.

The recovery percentages of napropamide including metabolites from the goats varied from 75.94 to 78.02 and excretion of the parent compound through feces varied from 18.86 to 21.59% indicating the majority of orally administered napropamide was absorbed. Hepatic clearance value (Cl_H) was three times higher than that of the renal clearance (Cl_R) value indicating some amount of parent compound was excreted into the intestine through bile. In addition, it is expected that some amount of the nonabsorbed part may undergo metabolism induced by protozoa and ruminal microflora present in the gastrointestinal tract and may be excreted as metabolites. Therefore, napropamide and its metabolites recovered from the feces of goats may be the result of both absorbed and nonabsorbed portions.

All tissues contained napropamide residues (except bile, testis, and uterus) after 4 and 5 days after dosing. Brain, rumen, spleen, fat, muscle, reticulum, omasum, small and large intestine, lung, rumen, and small intestinal content; while rumen, muscle, reticulum,

Table 2. Napropamide and Its Metabolites Recovered from Feces of Goats Following Oral Administration of Single High Dose at 2.5 g/kg[a]

	day 4		day 5		day 6		day 7	
time (hours)	P[b]	M[c]	P	M	P	M	P	M
0−24	1890 ± 130	118 ± 18	1724 ± 175	93 ± 13	2253 ± 300	188 ± 32	1955 ± 34	192 ± 46
24−48	3357 ± 328	609 ± 80	3277 ± 227	407 ± 62	3763 ± 333	805 ± 67	3500 ± 227	831 ± 110
48−72	187 ± 35	120 ± 26	159 ± 37	155 ± 36	241 ± 43	219 ± 35	199 ± 25	205 ± 45
72−96	57 ± 4	85 ± 11	42 ± 4	81 ± 10	70 ± 10	115 ± 19	72 ± 11	68 ± 11
96−120	−[d]	−	34 ± 10	52 ± 7	35 ± 5	42 ± 6	51 ± 10	50 ± 9
120−144	−	−	−	−	BDL[e]	29 ± 2	BDL	35 ± 2
144−178	−	−	−	−	−	−	BDL	BDL
total	5491	932	5236	788	6362	1398	5777	1381
recovery %	18.86	4.20	21.59	4.26	20.18	5.82	19.85	6.23
total recovery (P + M)	23.06		25.85		26.00		26.08	

[a] Mean value of 6 replicates with SE. [b]Napropamide recovered, in mg. [c]Metabolites recovered, in mg. [d](−), not available. [e]BDL, below detection limit.

Table 3. Napropamide and Its Metabolites Recovered from Urine of Goats Following Oral Administration of Single High Dose at 2.5 g/kg[a]

	day 4		day 5		day 6		day 7	
time (hours)	P	M	P	M	P	M	P	M
0−24	46 ± 5	1625 ± 180	58 ± 4	1552 ± 105	65 ± 7	1693 ± 96	39 ± 1	1714 ± 168
24−48	89 ± 11	6747 ± 503	105 ± 11	5504 ± 353	131 ± 16	8124 ± 388	76 ± 5	8527 ± 495
48−72	30 ± 7	467 ± 50	42 ± 5	580 ± 100	56 ± 2	706 ± 115	32 ± 5	323 ± 57
72−96	21 ± 2	200 ± 72	30 ± 1	246 ± 79	30 ± 3	320 ± 97	18 ± 1	287 ± 60
96−120	−[d]	−	BDL[e]	60 ± 10	BDL	42 ± 7	BDL	84 ± 18
120−144	−	−	−	−	BDL	6 ± 1	BDL	10 ± 1
144−178	−	−	−	−	−	−	BDL	BDL
total	186	9039	235	7942	282	10891	165	10945
recovery %	0.63	40.81	0.96	43.03	0.89	45.40	0.56	49.42
total recovery (P + M)	41.44		43.99		46.29		49.98	

[a] Mean value of 6 replicates with SE. [b]Napropamide recovered, in mg. [c]Metabolites recovered, in mg. [d](−), not available. [e]BDL, below detection limit.

omasum, abomasum, small and large intestine, rumen, and small intestinal content retained the residue of the parent compound after 6 and 7 days after dosing, respectively. Goats treated orally with a single dose of napropamide at 2.5 g kg^{-1} exhibited toxic symptoms such as piloerection, moderate bloat and drowsiness, and their increased rate and depth of respiration indicated the involvement of both peripheral and central nervous system. Therefore, presence of napropamide in the brain tissue of goat after 4 and 5 days of sacrifice is justified and well documented.

All tissues retained metabolites after 4, 5, 6 (except brain), and 7 (except brain, fat, and ovary) days of sacrifice. The maximum quantity of parent compound including metabolites was recovered from the different tissues of goat sacrificed on 4 days after dosing and then declined and negligible quantities were detected in some of the organs on 7 days after dosing.

Recovery of parent compound from the urine of goat varied from 0.63 to 0.96% and thereby corroborated the low renal clearance (Cl_B) value. However, the recovery percentages of napropamide including metabolites from the urine of goats varied from 41 to 49. Maximum quantity of parent and metabolites were recovered from urine of different groups at 48 h. Three major metabolites, viz, α-naphthoxy propionic acid (**2**), 1,5-dihydroxy naphthalene (**3**), and *N*-ethyl napropmide (**4**), were identified in feces, urine, and tissues. Napropamide is a powerful inducer of Cytochrome P_{450} content of liver microsomal tissue of goat and accordingly, it is expected that napropamide (**1**) undergoes amide hydrolysis, O-dealkylation, and N-dealkylation resulting in the formation of α-naphthoxy propionic acid, 1,5-dihydroxynaphthalene, and *N*-ethyl napropamide, respectively (Figure 3) in the animal system. The maximum amount of metabolites was recovered from urine, followed by tissue and feces in sequence. From the stoichiometric relation, it appears that the recovery percentages of metabolites in terms of parent compound from urine and tissues are multiple-times higher than that of parent compound. Oral toxicokinetic values of napropamide like *Vd* (area), *Kel*, and K_{12} indicated that napropamide, upon reaching blood, is rapidly distributed into the tissues and subsequently withdrawn in blood. The present experiment has not been set for the kinetic study of metabolites, but it is expected that metabolites (similarly to the parent compound) are rapidly distributed into tissues

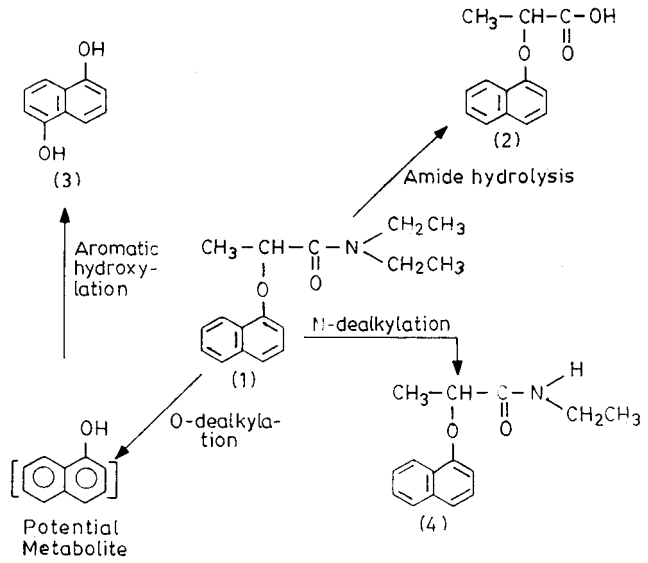


Figure 3. Probable metabolic pathways of napropamide.

Table 4. Napropamide and Its Metabolites Recovered from Tissues of Goats Following Oral Administration of Single High Dose at 2.5 g/kg

	day 4		day 5		day 6		day 7	
tissue	P[b]	M[c]	P	M	P	M	P	M
liver	1.21 ± 0.00	29.50 ± 2.76	0.98 ± 0.02	14.77 ± 2.06	BDL[d]	4.11 ± 0.52	BDL	3.95 ± 0.94
kidney	0.24 ± 0.00	24.50 ± 7.69	1.16 ± 0.00	11.20 ± 1.68	BDL	3.48 ± 0.21	BDL	4.02 ± 0.90
heart	0.83 ± 0.02	39.50 ± 2.92	0.64 ± 0.01	29.02 ± 3.77	BDL	9.77 ± 1.21	BDL	4.43 ± 1.40
brain	1.64 ± 0.01	52.50 ± 4.75	1.21 ± 0.01	14.77 ± 1.23	0.60 ± 0.01	BDL	BDL	BDL
rumen	7.24 ± 0.05	117.80 ± 9.34	6.30 ± 0.05	77.44 ± 20.30	3.09 ± 0.00	42.75 ± 7.50	1.50 ± 0.00	34.23 ± 7.18
spleen	0.05 ± 0.00	133.50 ± 8.61	0.04 ± 0.01	75.18 ± 18.77	0.02 ± 0.00	42.38 ± 4.82	BDL	50.50 ± 0.78
fat	6.08 ± 0.52	77.10 ± 9.92	6.00 ± 1.15	44.62 ± 5.04	3.01 ± 0.00	42.23 ± 6.03	BDL	BDL
muscle	32.96 ± 7.58	76.00 ± 7.44	24.49 ± 2.25	55.32 ± 10.24	10.05 ± 1.19	25.23 ± 1.00	1.05 ± 0.00	20.50 ± 5.00
bile	0.10 ± 0.00	127.00 ± 12.37	BDL	95.59 ± 8.35	BDL	55.25 ± 2.95	BDL	49.62 ± 3.50
reticulum	4.24 ± 0.58	154.50 ± 18.59	2.99 ± 0.00	87.02 ± 7.75	2.00 ± 1.05	76.53 ± 4.38	0.95 ± 0.00	55.80 ± 8.76
omasum	7.96 ± 2.06	88.70 ± 13.04	5.43 ± 0.02	30.67 ± 1.85	2.14 ± 0.00	13.16 ± 2.00	0.50 ± 0.11	4.65 ± 1.10
abomasum	2.32 ± 0.00	92.80 ± 7.25	1.15 ± 0.13	30.29 ± 2.62	1.74 ± 0.00	17.21 ± 1.00	0.78 ± 0.10	16.00 ± 1.12
small intestine	8.50 ± 2.01	106.90 ± 11.09	6.70 ± 0.06	132.29 ± 1.62	2.50 ± 0.01	55.36 ± 5.25	0.52 ± 0.00	30.50 ± 18.09
large intestine	7.80 ± 1.60	82.60 ± 5.92	5.81 ± 1.01	37.17 ± 0.00	3.48 ± 0.54	27.98 ± 1.15	1.32 ± 0.02	26.16 ± 1.23
ovary	0.03 ± 0.00	25.40 ± 1.85	BDL	10.49 ± 1.00	BDL	1.04 ± 0.00	BDL	BDL
uterus	0.02 ± 0.00	42.60 ± 3.01	BDL	11.02 ± 1.00	BDL	4.62 ± 1.00	BDL	2.09 ± 0.00
skin	0.84 ± 0.10	85.80 ± 4.10	0.75 ± 0.00	52.01 ± 3.55	BDL	35.19 ± 3.90	BDL	30.66 ± 0.00
adrenal gland	0.00 ± 0.00	1.02 ± 3.50	0.00 ± 0.00	0.43 ± 0.07	BDL	0.17 ± 0.00	BDL	0.10 ± 0.10
lung	1.20 ± 0.00	71.90 ± 5.37	1.01 ± 0.00	66.09 ± 11.88	0.54 ± 0.00	20.15 ± 3.48	BDL	16.50 ± 1.71
bone	0.00 ± 0.00	102.50 ± 4.76	0.00 ± 0.00	40.09 ± 2.00	BDL	15.20 ± 1.13	BDL	3.04 ± 0.00
testis	0.00 ± 0.00	115.50 ± 2.25	BDL	46.42 ± 8.25	BDL	22.00 ± 2.82	BDL	14.91 ± 1.00
rumen content	384.16 ± 223.52	156.80 ± 1.60	301.29 ± 10.00	98.83 ± 2.63	100.56 ± 23.00	37.65 ± 5.14	34.50 ± 2.01	40.50 ± 3.01
large intestine content	67.41 ± 11.56	323.74 ± 33.54	62.91 ± 5.20	199.53 ± 18.00	BDL	93.15 ± 16.67	BDL	67.50 ± 3.99
small intestine content	28.56 ± 3.54	331.20 ± 26.05	26.11 ± 3.00	220.58 ± 22.38	19.93 ± 2.00	121.01 ± 8.05	1.39±0.00	92.50±4.39
total	563.40	2459.36	454.97	1480.84	149.66	769.10	42.51	568.16
recovery %	1.93	11.59	1.87	8.37	0.47	3.18	0.14	2.68
total recovery (P + M)	13.52		10.24		3.65		2.82	

[a] Mean value of 6 replicates with SE. [b] Napropamide recovered, in mg. [c] Metabolites recovered, in mg. [d] BDL, below detection limit.

Table 5. Total Recovery Percentage of Napropamide and Its Metabolites from Goats Sacrified on Different Days Following Oral Administration of Single High Dose at 2.5 g/kg

	day 4		day 5		day 6		day 7	
substrate	P	M	P	M	P	M	P	M
feces	18.86	4.20	21.59	4.26	20.18	5.82	19.85	6.23
urine	0.63	40.81	0.96	43.03	0.89	45.40	0.56	49.42
tissues	1.93	11.59	1.87	8.37	0.47	3.18	0.14	2.68
recovery %	21.42	56.6	24.42	55.66	21.54	54.40	20.55	58.33
total recovery (P + M)	78.02		80.08		75.94		78.88	

Table 6. Effect of Napropamide on Cytochrome P_{450} Contents of Liver Microsomal Pellet of Goats Following Repeated Oral Administration at 500 mg kg^{-1} for 7 Days[a]

	animals				mean with	
	1	2	3	4	SE	F value
group 1 (control)[b]	0.98	1.07	1.12	1.13	1.07 ± 0.03	
group 2[b]	2.37	2.88	3.30	3.12	2.92 ± 0.02	34.22**
group 3[c]	2.27	2.30	2.14	2.20	2.22 ± 0.04	

[a] Cytochrome P_{450} results reported as n mole mg^{-1} of mocrosomal protein. [b] Goats slaughtered after 7 days. [c] Goats slaughtered after 5 days. $^{**}P < 0.01$ compared to control.

followed by withdrawal which then slowly excretes through urine for a long time. Simultaneously, the metabolites (such as parent compound) may also excrete through bile and continue to be excreted through feces persistently for a longer period. The material balance of herbicide including metabolites after single-dose oral administration (75.94 to 80.08%) seems to be quite satisfactory.

ACKNOWLEDGMENT

We acknowledge the gift of technical grade napropamide and its metabolites from M/S Gharda Chemicals Ltd., Mumbai.

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Received for review December 20, 1999. Revised manuscript received November 27, 2000. Accepted December 1, 2000. Financial assistance was provided by M/S Gharda Chemicals Ltd., Mumbai, India.

JF991387W