Metabolic Study of Isoproturon in Goats Following a Single Oral Administration: Toxicokinetics and Recovery

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Toxicokinetic behavior and recoveries of isoproturon from feces, urine, and different tissues of goat were determined after 4, 5, 6, and 7 days following single oral administration at 500 mg/kg. Isoproturon was rapidly absorbed and attained blood concentration within 15 min of administration. The kinetic behavior followed a two-compartment open model. The higher $t_{1/2}(\beta)$ (9.78 ± 0.33 h) and $V_{d_{area}}$ (4.49 ± 0.41 L/kg) associated with lower Cl_B (0.32 ± 0.02 L/kg/h) suggested slow elimination from the blood. Approximately 56% of the total administered compound was recovered from feces. The rate of excretion of isoproturon through feces was maximum at 48 h and could not be detected beyond 120 h. The excretion pattern of isoproturon through urine resembled that of feces, and approximately 10−11% was eliminated in urine. A maximum quantity of residue was detected in all tissues of goats slaughtered after 4 days followed by a substantial decline after day 5, and nothing could be detected after day 7. Histopathological study revealed that isoproturon produced moderate cellular changes like fatty degeneration in the liver and kidney and emphysema in the lung after 7 days post administration.

Keywords: *Toxicokinetics; recovery; isoproturon; goat; histopathology*

INTRODUCTION

Isoproturon, *N*,*N*-dimethyl-*N*′-(4-isopropylphenyl)urea (Figure 1), is a member of the family of substituted ureas. It has been developed as an effective herbicide and is widely used for postemergency application in wheat crops (Anon, 1977). The toxicity of related substituted ureas, viz. linuron, diuron, fenuron, and monuron, has been studied in rats and dogs (Hodge et al., 1968; Geissbiihler et al., 1975) and sheep and cattle (Palmer and Radeleff, 1964) and reported to have a low order of toxicity. Rakha et al. (1983) clinically studied the toxic effects of isoproturon in sheep, goat, cattle, and buffalo after consumption of freshly sprayed fodder and spraying over the body and reported that the herbicide produced identical toxic symptoms like that of fenuron, monuron, and other members of the family.

It is expected that necessary data in respect to the rate of absorption, distribution, and retention in tissues of the compound should be generated in an animal system before it is widely used and declared safe to avoid possible health hazards. However, from the above reports, the distribution pattern and as well as the residual retention of isoproturon in animal tissues are not clear and warrant further study in animal systems. Goat is considered as the model animal of study since its meat is popularly consumed by human beings. Therefore, the present experiment studied the toxicokinetic behavior of isoproturon and its recovery from feces, urine, and in different tissues at different time intervals following single oral administration.

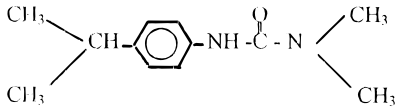

Figure 1. Structure of isoproturon.

EXPERIMENTAL PROCEDURES

Chemicals. Isoproturon (Avanon), technical grade, was supplied by M/S Gharda Chemical Ltd., Bandra, Bombay. The purity of the compound was 97.3%. All the chemicals and solvents used in this study was obtained from E. Merck (India), AR grade.

Animal Treatment. Clinically healthy adult black Bengal female (nulliparous) and male goats weighing between 9.5 and 12 kg were considered. The goats were acclimated individually in custom made stainless steel metabolism cages (48 in. × 48 in. × 36 in.) for a period of 15 days, and within the period they were dewormed with levamisole at a rate of 7.5 mg/kg. The animal house was provided with artificial lighting and controlled temperature (22 ± 3 °C). The goats were fed with standard feed (two parts wheat husk, one part groundnut cake, one part crushed gram, one part crushed maize, and two parts green) and had free access to fresh water. Each animal was fasted overnight before administration of vehicle and or isoproturon.

For ascertaining minimum oral toxic dose level, four different dose levels of isoproturon after suspension in (carboxymethyl)cellulose (1% w/v) were administered to four groups of goats separately, each comprising one male and female.

For the recovery study of isoproturon, 13 male and 14 female goats were taken, out of which one male and two females were

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Scheme 1. Extraction and Analysis of Caprine Feces

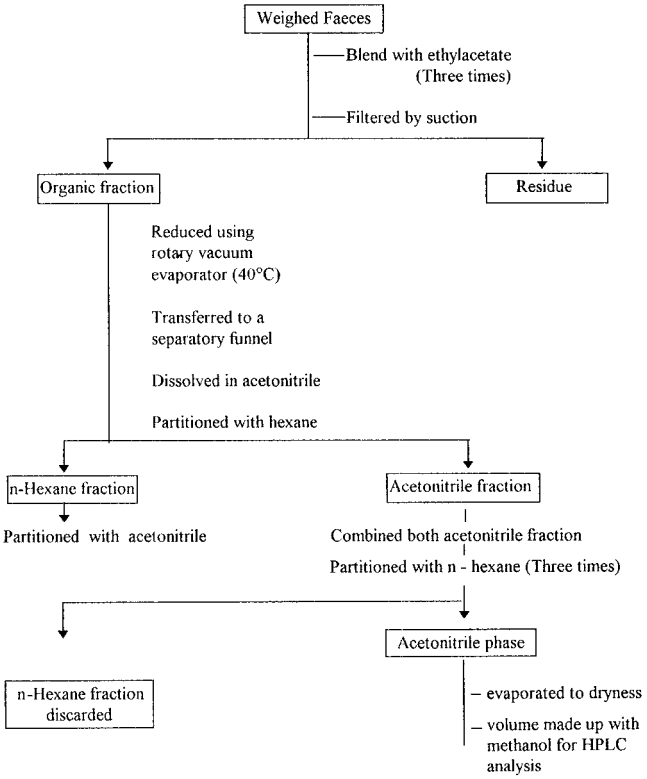


Table 1. Kinetic Parameters for Isoproturon[a] after a Single Oral Administration to Goats at 500 mg kg^{-1} (n = 5, Mean ± SE Values of both Male and Female)

param	value
K_a (h^{-1})	1.38 ± 0.24
$t_{1/2}$ (K_a) (h)	0.53 ± 0.08
β (h^{-1})	0.071 ± 0.002
$t_{1/2}$ (β) (h)	9.78 ± 0.33
T−P	0.95 ± 0.09
$V_{d_{area}}$ (L kg^{-1})	4.49 ± 0.41
AUC (μg h mL^{-1})	1597.22 ± 142.06
K_{el} (h^{-1})	0.14 ± 0.007
Cl_B (L kg^{-1} h^{-1})	0.32 ± 0.02
Cl_R (L kg^{-1} h^{-1})	0.06 ± 0.01
Cl_H (L kg^{-1} h^{-1})	0.26 ± 0.03

[a] Test chemical.

kept as control and received vehicle only. The remaining male and female goats were divided into four equal groups, each comprising of three male and female animals. The first, second, third, and fourth groups received isoproturon orally after suspension in (carboxymethyl)cellulose (1% w/v) and were sacrificed after 4, 5, 6, and 7 days, respectively. For the kinetic study, three male and two female goats of the first group (treatment) were considered.

Minimum Oral Toxic Dose. Isoproturon at four dose levels in ascending order, i.e., 100, 200, 400, and 500 mg/kg, after suspension in (carboxymethyl)cellulose solution (1% w/v) was administered once orally to one animal of different groups, respectively. They were then kept under clinical observation. Goats treated with 100, 200, and 400 mg kg^{-1} of isoproturon did not show any clinical sign of toxicity, while goats treated with 500 mg kg^{-1} of isoproturon showed the following symptoms: depression within 6 h and grinding of teeth, nasal discharge, hyperthermia by 4 day pd. Anorexia started within 4 h and continued until 48 h. Bloat developed by 8 h and persisted until 36 h. The frequency of urination was reduced by 36 h. Clinical routine examination of urine did not reveal the presence of blood, glucose, albumen, bile salts, etc. Animals were relieved of the above symptoms by 96 h. Henceforth, this dose level was selected as the minimum oral toxic dose in the present study.

Table 2. Residue of Isoproturon[a] (ppm) in Caprine Tissues at Different Days Following a Single Oral Administration at 500 mg kg^{-1} (n = 6; Mean ± SE Values of both Male and Female)

	isoproturon recovered (ppm)			
tissue	4 days	5 days	6 days	7 days
liver	2.15 ± 0.23	5.86 ± 0.78	BDL	BDL
kidney	0.54 ± 0.08	0.51 ± 0.13	BDL	BDL
lung	1.15 ± 0.16	4.08 ± 0.58	BDL	BDL
heart	6.80 ± 0.20	10.0 ± 0.98	BDL	BDL
muscle	2.14 ± 0.53	0.125 ± 0.02	BDL	BDL
spleen	0.72 ± 0.14	1.61 ± 0.35	BDL	BDL
fat	56.0 ± 7.60	5.77 ± 0.34	BDL	BDL
adrenal gland	9.82 ± 0.86	24.46 ± 4.70	BDL	BDL
ovary (3) (a)	10.38 ± 1.04	110.47 ± 27.21	BDL	BDL
testis (3) (b)	1.6 ± 0.02	2.59 ± 0.26	BDL	BDL
uterus (3) (a)	37.5 ± 2.0	14.55 ± 0.04	BDL	BDL
bile	2666.7 ± 188.8	717.50 ± 110.0	BDL	BDL
skin	28.11 ± 6.21	1.73 ± 0.20	BDL	BDL
intestine	0.74 ± 0.06	8.82 ± 0.17	4.12 ± 0.63	BDL
forestomach rumen reticulum omasum abonasum	15.21 ± 0.005	27.56 ± 6.05	BDL	BDL
rumen content	131.2 ± 5.0	49.56 ± 9.97	29.6 ± 2.95	BDL
bone	10.03 ± 1.45	2.45 ± 0.33	BDL	BDL
brain	BDL	BDL	BDL	BDL
blood	3.00	BDL	BDL	BDL

[a] Test chemical; BDL, below detection limit (<0.01 ppm); number in parentheses indicates number of goats, (a) female, (b) male.

Analytical Procedures. *Kinetics.* For kinetic study, blood samples (1 mL) were collected from the jugular vein into heparinized tubes before and after 15, 30, 45 min and 1, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72, and 96 h of the isoproturon administration. The concentration of isoproturon in the blood was estimated by HPLC. The values of kinetic parameters like, C^B_{max}, C^B_{min}, K_a, $t_{1/2}$ (K_a), β, $t_{1/2}$ (β), T−P, $V_{d_{area}}$, AUC, K_{el}, Cl_B, Cl_R, and Cl_H were determined from semilogarithmic plots of blood level time profile data in goats using standard formulas (Baggot, 1977).

Collection of Feces and Urine and Extraction. Feces and urine of individual goat were collected at 24, 48, 72, 96, 120, 148, and 168 h pd. The excretions were measured or weighed and stored at −20 °C prior to extraction. The method of extraction from feces (5 g) is presented in Scheme 1.

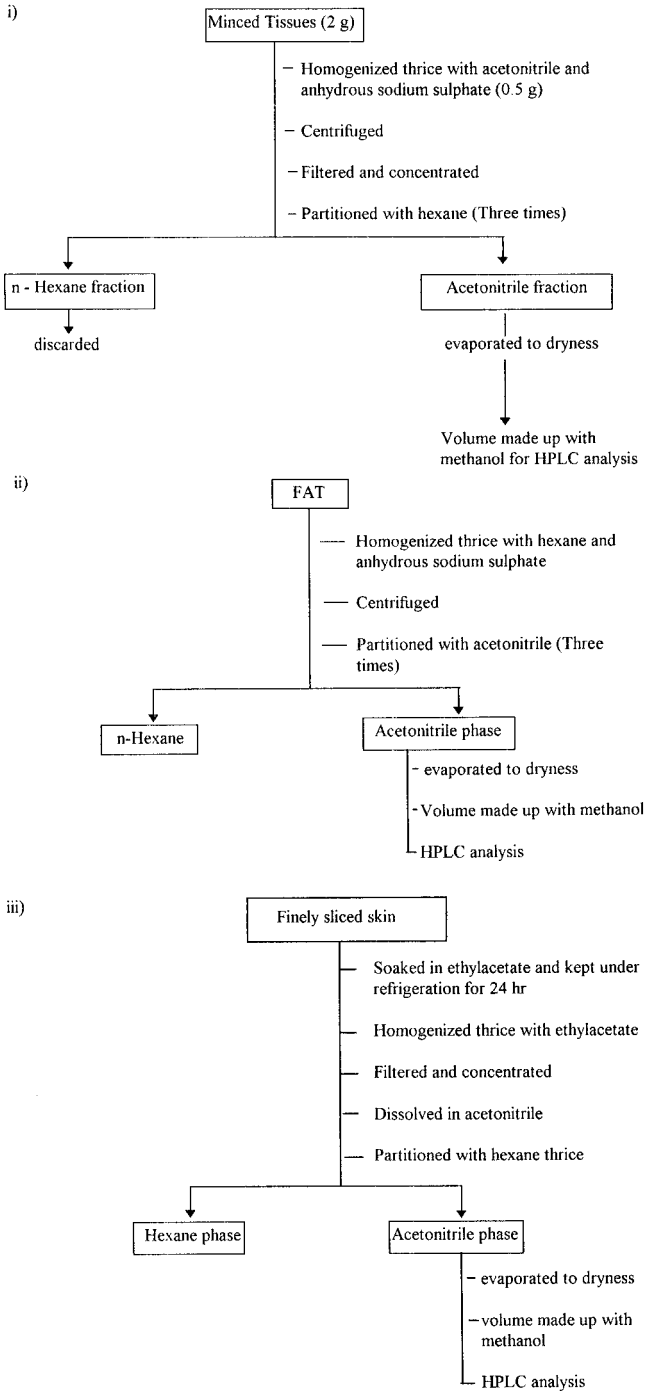
To each urine sample was added an equal volume of distilled water, and the urine was extracted three times with an equal volume of diethyl ether (50 mL × 3). The ether fraction was passed through anhydrous sodium sulfate (~4 g) and evaporated to dryness and the final volume made up with methanol for HPLC analysis.

Tissue Extraction and Quantification. The animals of the first, second, third, and fourth groups were killed after 4, 5, 6, and 7 days pd, respectively. Samples of liver, kidney, lungs, brain, heart, spleen, adrenal gland, thigh muscle, omental fat, ovary, uterus, testis, rumen, reticulum, omasum, abomasum, intestine, skin, bone, and contents of rumen and intestine were taken, weighed, chopped, and stored at −20 °C prior extraction (Scheme 2, i−iii). The same procedure of extraction and quantification was followed for crushed bone as skin except for the homogenization step.

Extraction and quantification of intestinal and ruminal contents were done in the same way as described for feces extraction.

Blood, Bile Extraction, and Quantification. To sample of blood, bile (1 mL), saturated ammonium sulfate in 2.5% sulfuric acid (2.5 mL), and ethyl acetate (20 mL) were added and the mixture shaken and filtered through anhydrous sodium sulfate (~4 g). The test tubes were washed three times with equal volume of ethyl acetate (20 mL). The filtrate was evaporated to dryness using a rotary vacuum evaporator at 40 °C, and the final volume was made up with methanol (1 mL) for HPLC analysis.

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



For histopathological examination, a portion of liver, kidney, heart, spleen, and lung specimens from the control and experimental groups of goats was removed, fixed in formalin (10%), processed by using standard paraffin embedding, stained with hematoxylin and eosin, and then examined under a microscope (Lillie and Fullmer, 1976).

HPLC. A Hewlett-Packard Model 1050 liquid chromatography coupled with a variable wavelength UV/vis detector attached to a 3392A integrator was used for the analysis of isoproturon. The operational parameters were as follows: mobile phase, acetonitrile:water (1:1), the mixture was subject to membrane filtration and degassed by ultrasonification; flow, 1.5 mL min^{-1}, column reversed-phase (μ-Bondpack C_{18}); injection using Hamilton Microlitre syringe (25 μL); detector wavelength, 240 nm.

Chromatographic Procedures. A stock solution of 100 mg L^{-1} of isoproturon (technical grade, 97.3%) was prepared in methanol as standard. The retention time of isoproturon was 3.64 min (Figure 2). The retention time of the parent compound occurring in blood/tissue/urine/feces was compared with that of the external standard, and the data were recorded in a HP 3392A integrator.

Recovery. The recoveries of isoproturon were estimated by adding known quantities of isoproturon to give final concentrations of 0.15, 0.45, 0.60, and 0.90 ppm for blood and urine and 0.75, 2.25 and 4.5 ppm for feces and other tissues. The area of HPLC peaks against several concentrations of isoproturon was plotted, and linearity was found to be maintained. The linearity of the calibration curve was checked. The recoveries of isoproturon from blood/urine and other substrates varied from 83 to 94%. The limit of detection of isoproturon was 0.01 ppm.

Statistical Analysis of Data. Mean and standard error (SE) of the data were calculated by using standard formulas (Snedecor, 1959).

RESULTS

Isoproturon in Blood. Isoproturon was detected in blood at 15 min (30.33 ± 6.06 μg mL^{-1}), maximum (C^B_{max} level, 90.00 ± 7.64 μg mL^{-1}) at 2 h, and thereafter the concentration declined (C^B_{min} value, 19.33 ± 1.86 μg mL^{-1}) until 24 h, after which time isoproturon could not be detected (Figure 3). The kinetic behavior followed a two-compartment open model. The $V_{d_{area}}$, β, $t_{1/2}$ (β), K_a, $t_{1/2}$ (K_a), and T−P of isoproturon were 4.49 ± 0.41 L kg^{-1}, 0.07 ± 0.002 h^{-1}, 9.78 ± 0.33 h, 1.38 ± 0.24 h^{-1}, 0.53 ± 0.08 h, and 0.95 ± 0.09, respectively, while AUC, Cl_B, Cl_R, and Cl_H values were, respectively, 1597.22 ± 142.06 μg h mL^{-1}, 0.32 ± 0.02 L kg^{-1} h^{-1}, 0.06 ± 0.01 L kg^{-1} h^{-1}, and 0.26 ± 0.03 L kg^{-1} h^{-1} (Table 1).

Recovery of Isoproturon. *Feces.* The rate of excretion of isoproturon through feces was initially slow followed by a maximum at 48 h and thereafter declined gradually by 96 h and completed by 120 h. Isoproturon could not be detected beyond 120 h in the feces of any group of goats (Table 3). The percentages recovered from feces of goats slaughtered after 4, 5, 6, and 7 days of administration were 57.02, 57.63, 56.20, and 57.98, respectively (Table 5).

Urine. The excretion pattern of isoproturon through urine resembled that of feces. The rate of excretion was initially slow, reaching a peak at 48 h and thereafter declining gradually. The lowest rate was recorded at 96 h, and nothing could be detected beyond 96 h (Table 4). The recovery percentages were 5.04, 9.35, 10.41, and 9.59 from the urine of goats sacrificed after 4, 5, 6, and 7 days, respectively (Table 5).

Tissues. The maximum residue of isoproturon was detected in all the tissues except the brain of goats slaughtered after 4 days followed by a substantial decline after 5 days. However, residual concentration could be detected only in the intestine and rumen contents after 6 days (Table 2). Percentages of recovery of isoproturon from tissues of goats slaughtered after 4, 5, and 6 days were 8.00, 2.54, and 1.55, respectively (Table 5).

Histopathology. Histopathology examination of liver after 4 and 7 days showed fatty degeneration (Figure 4 top, middle). Sections of kidney showed fatty degeneration of tubules (Figure 5 top) and fatty vacuolation in the glomerulus after 7 days of administration. Likewise, lung exhibited the presence of emphysema (Figure 6 top) and evidence of accumulation of oedematous fluid (Figure 6 middle).

Table 3. Recovery of Isoproturon[a] (ppm) from Caprine Feces at Different Time Intervals Following Single Oral Administration at 500 mg kg^{-1} (n = 6; Mean ± SE Values of both Male and Female)

	isoproturon recovered (ppm)			
time in (h)	4 days	5 days	6 days	7 days
0−24	170 ± 0.60	1955.37 ± 167.08	1699.83 ± 114.69	2223.64 ± 173.23
24−48	7620.6 ± 726.95	11043.48 ± 796.04	10017.18 ± 427.05	11477.14 ± 346.67
48−72	3104.79 ± 239.58	622.6 ± 53.65	469.32 ± 44.86	633.91 ± 38.69
72−96	1042.86 ± 123.67	138.36 ± 29.98	174.52 ± 34.30	233.3 ± 44.38
96−120		83.3 ± 21.45	75.52 ± 18.71	150 ± 35.1

[a] Test chemical.

Table 4. Recovery of Isoproturon[a] (ppm) from Caprine Urine at Different Time Interval Following Single Oral Administration at 500 mg kg^{-1} (n = 6, Mean ± SE Values of both Male and Female)

	isoproturon recovered (ppm)			
time (h)	4 days	5 days	6 days	7 days
0−24	114.6 ± 6.23	371.81 ± 30.95	216.54 ± 18.75	355.76 ± 25.30
24−48	522.67 ± 45.65	1037.88 ± 31.39	1172.79 ± 102.29	1063.75 ± 39.16
48−72	26.62 ± 4.62	131.15 ± 10.49	120.74 ± 20.67	152.79 ± 12.98
72−96	12.93 ± 1.72	56.98 ± 8.92	40.74 ± 7.18	55.29 ± 12.78
96−120		BDL	BDL	BDL

[a] Test chemical; BDL, below detection limit (<0.01 ppm).

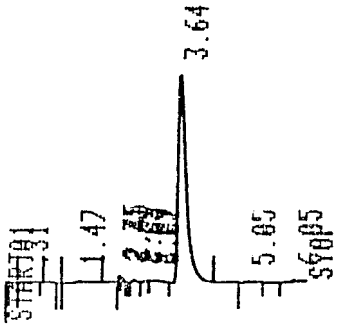


Figure 2. Chromatogram of isoproturon (analytical standard).

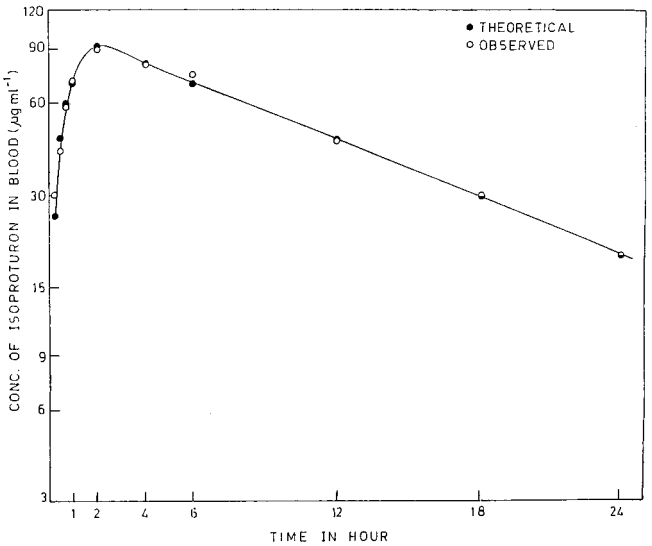


Figure 3. Semilogarithmic plot of observed (○) and theoretical (●) concentration of isoproturon in goat blood against time after a single oral dose of 500 mg kg^{-1} (n = 5).

DISCUSSION

Isoproturon in blood was detected at 15 min and persisted for 24 h. The absorption rate constant (K_a) indicated rapid absorption from the gastrointestinal tract of goat, despite the fact that the total percentage of isoproturon recovered from feces exceeded 56%. The higher Cl_H value and residual level in bile indirectly suggested that the major route of elimination of absorbed isoproturon might be directed through liver. Therefore, the total percentage recovered from feces of goats slaughtered on different days were the sum values of the unabsorbed and a part of the absorbed portion of isoproturon. Comparatively, the percentages of recovery from urine as well as residual level in kidney tissue were found to be meager and supplemented the observed low Cl_R value. Administration of isoproturon through the oral route might have been a possible reason for retention of the maximum residue in the ruminal content. All the tissues except brain and blood retained the residue after 4 days of oral administration, indicating wide distribution of the compound in the body and corroborated the higher AUC value. The higher $t_{1/2}$ (β) and $V_{d_{area}}$ associated with lower Cl_B suggest slow elimination of the herbicide from the blood.

Table 5. Recovery Percentage of Isoproturon[a] on Different Days Following a Single Oral Administration at 500 mg kg^{-1} in Goat (n = 6, Mean Values of both Male and Female)

	% recovery			
substrate	4 days	5 days	6 days	7 days
feces	57.02	57.63	56.2	57.96
urine	5.04	9.35	10.41	9.59
tissues	8.00	2.54	1.55	BDL
total	70.06	69.52	68.16	67.57

[a] Test chemical; BDL, below detection limit (<0.01 ppm).

Only 70% of the administered isoproturon (test chemical) was recovered from the goats slaughtered after 4 days, and after that recovery percentages were progressively reduced along with time. It is expected that some portion of isoproturon undergoes metabolism by this time and some quantity remains in remnant parts of carcass that have not been analyzed.

Oral administration of isoproturon showed the symptoms of central nervous system (CNS) depression, though no residue could be detected in the brain tissue after 4 days. On the other hand, symptoms like formation of bloat and lesser frequency of urination and defecation suggested possible involvement of the cholinergic system in addition to CNS. Sarkar and Gupta (1993) also reported that isoproturon had an inhibitory effect on central motor performance and sedative action on CNS of rat. Since no residual study of isoproturon

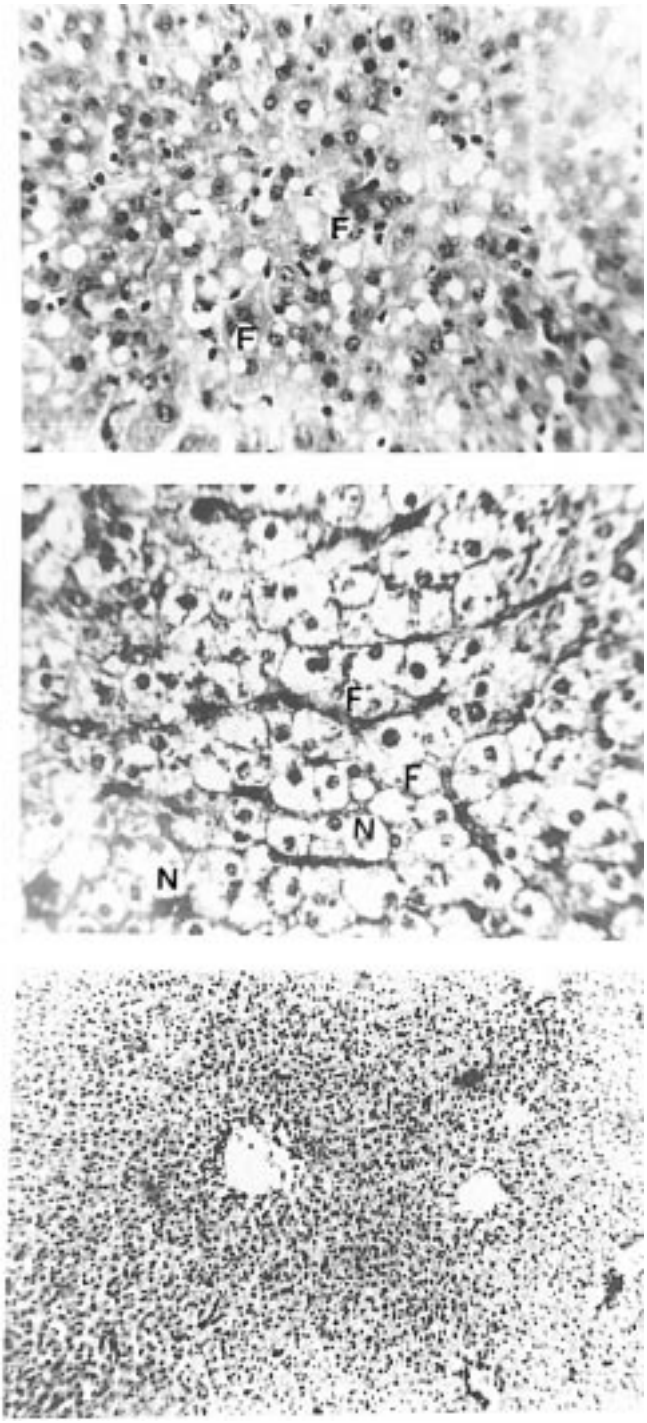


Figure 4. (Top) section of liver showing fatty degeneration (F) after 4 days of single oral administration of isoporturon at 500 mg kg^{-1} in goat H & E 45 × 10. (Middle) section of liver showing fatty degeneration (F) after 7 days of single oral administration of isoproturon at 500 mg kg^{-1} in goat H & E 45 × 10. (Bottom) section of normal goat liver tissue H & E 10 × 10.

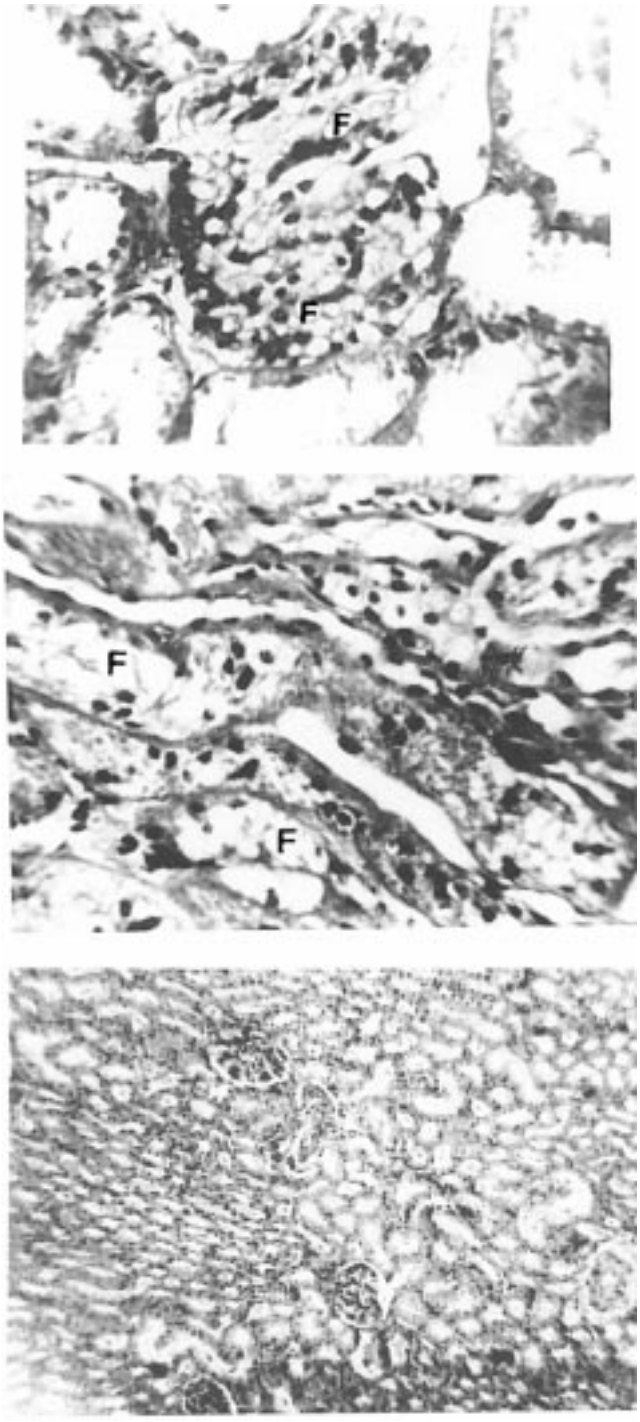


Figure 5. (Top) section of kidney showing fatty vacuolaton in the glomerulus (F) after 7 days of single oral administration of isoproturon at 500 mg kg^{-1} in goat H & E 45 × 10. (Middle) section of kidney showing fatty degeneration (F) of tubules after 7 days of single oral administration of isoproturon at 500 mg kg^{-1} in goat H & E 45 × 10. (Bottom) section of normal goat kidney tissue H & E 10 × 10.

in brain had been made at the time of depression, it becomes difficult to explain the possible reason for such involvement. Isoproturon did not persist more than 5 days in the omental fat, suggesting minimum lipophilicity.

The intensity of cellular changes that have been observed in liver, kidney, and lung under histopathological observation cannot be correlated with the quantitative presence of isoproturon. Both kidney and lung contained a sufficient quantity of residue after day 4, yet it failed to produce cellular alterations, whereas after day 7 the sections of liver, kidney, and lungs showed slight cellular alterations in the absence of residue.

ABBREVIATIONS USED

AUC, total area under curve; β, elimination rate constant; BDL, below detection limit; C^{B}_{max}, maximum blood concentration; C^{B}_{min}, minimum blood concentra-

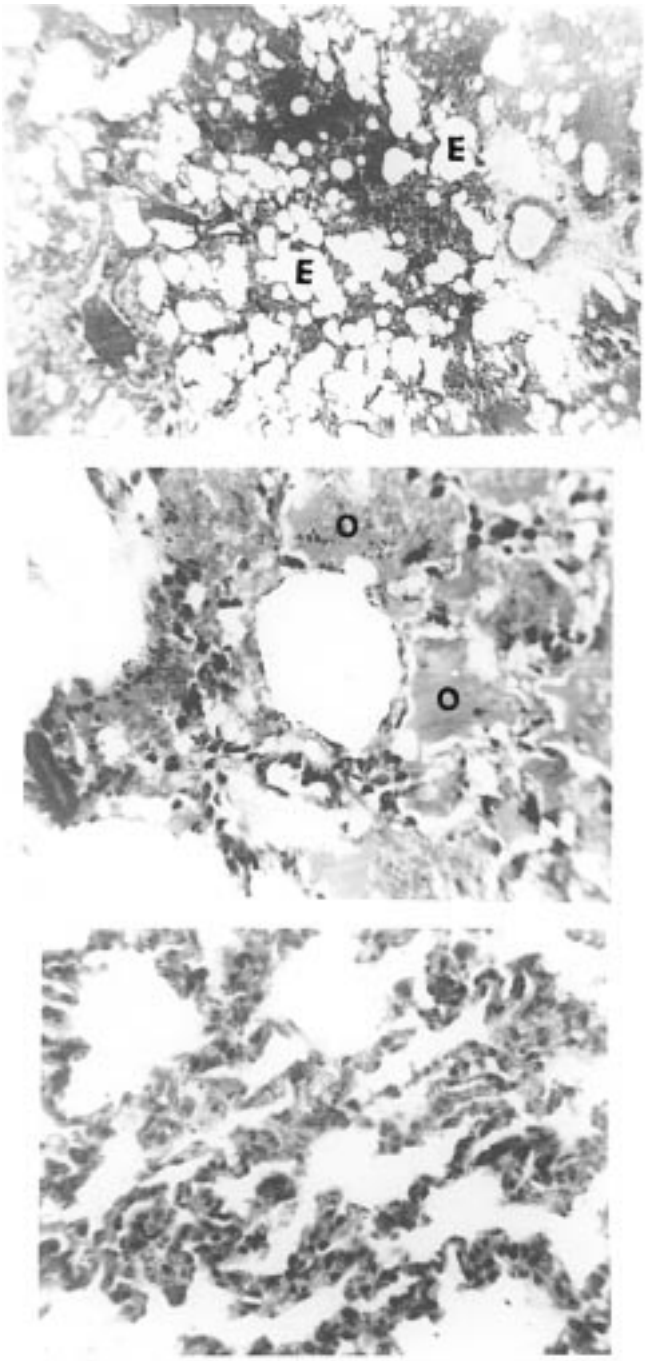


Figure 6. (Top) section of lung showing presence of emphysema (E) after 7 days of single oral administration of isoproturon at 500 mg kg^{-1}. (Middle) section of lung showing evidence of accumulation of oedematous fluid (O) after 7 days of single oral administration of isoproturon at 500 mg kg^{-1} in goats H & E 45 × 10. (Bottom) section of normal goat lung tissue H & E 45 × 10.

tion; Cl_R, renal clearance; Cl_B, total body clearance; Cl_H, hepatic clearance; H & E, hematoxylin and eosin; K_a, absorption rate constant; K_{el}, first-order rate constant for pesticide elimination from central compartment; pd, post administration; SE, standard error; T−P, tissue to plasma ratio; $t_{1/2}$ (K_a) absorption half-life; $t_{1/2}$ (β), elimination half-life; $V_{d_{area}}$, apparent volume of distribution according to area method.

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