



International Journal of Veterinary Sciences and Animal Husbandry



ISSN: 2456-2912

NAAS Rating (2026): 4.61

VET 2025; SP-11(4): 248-252

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www.veterinarypaper.com

Received: 17-01-2026

Accepted: 22-02-2026

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Toxicokinetic evaluation of alachlor in goats: Elimination kinetics, tissue distribution and recovery analysis using gas chromatography

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DOI: <https://www.doi.org/10.22271/veterinary.2026.v11.i4Sd.3230>

Abstract

Alachlor, a widely used chloroacetanilide herbicide, poses potential risks to animal health due to its toxicokinetic and metabolic profile. This study investigates the toxicokinetic of Alachlor in goats following oral administration at a dose of 400 mg/kg body weight. The concentration of Alachlor in plasma was analysed using gas chromatography. The pharmacokinetic parameters revealed a rapid distribution phase with a half-life ($t_{1/2K}$) of 54.12 ± 8.94 mins and an elimination half-life ($t_{1/2K_a}$) of 8.75 ± 2.49 . The volume of distribution ($V_{d\text{area}}$) was estimated at 1.76 ± 0.49 L/kg, indicating extensive tissue distribution. The total body clearance (CL_B) was determined to be 0.016 ± 0.002 L/Kg/min. The toxicokinetic profile indicates that Alachlor is rapidly metabolized and eliminated, but its bioaccumulation potential and toxic effects warrant further investigation. This study provides critical insights into the pharmacokinetics of Alachlor in goats, contributing to a better understanding of its safety and environmental impact in livestock-exposed regions.

Keywords: Alachlor, herbicide, toxicokinetic, gas chromatography, bioaccumulation

Introduction

India, with a population of approximately 1.44 billion in 2023, has surpassed China as the most populous country. Economic growth and urbanization are driving changes in dietary needs, necessitating sustainable agricultural practices. Agrochemicals, including herbicides, play a vital role in crop protection, with herbicide use increasing significantly from 15 tonnes in 1969-70 to 52,555 tonnes in 1991-92. Despite India's low pesticide consumption (0.6 kg/ha), herbicide use is rising due to intensive farming (Majumder, 1994) ^[10].

Although pesticides mitigate crop loss, they pose ecological risks, including bioaccumulation and cytotoxic effects (Köhler and Triebkorn, 2013) ^[8]. Herbicides such as glyphosate and chlorpyrifos adversely impact non-target species, including earthworms (Casabe *et al.*, 2007) ^[2], butterflies (Russell and Schultz, 2009) ^[15], and amphibians (Relyea and Hovermann, 2008) ^[13]. Goats, widely reared in India, frequently graze near agricultural fields and risk herbicide exposure, yet systematic investigations are lacking (FAOSTAT, 2008) ^[6].

Alachlor, a widely used herbicide, inhibits protein synthesis and root elongation. Its mutagenic and carcinogenic potential has been documented (Dearfield *et al.*, 1999) ^[5]. Studies have examined alachlor metabolism in humans (Coleman *et al.*, 1999) ^[3], its impact on fish antioxidants (Yi *et al.*, 2007) ^[17], and poisoning cases (Ying-Chun *et al.*, 2007) ^[18], but data on ruminants remain scarce. Given the economic importance of goats, this study aims to determine the toxicokinetic parameters of Alachlor in goats, focusing on its elimination kinetics, tissue distribution, and recovery over time. By utilizing gas chromatography, this study seeks to provide a comprehensive understanding of how Alachlor is absorbed, distributed, metabolized, and eliminated from the goat's body, along with an assessment of its recovery potential.

Materials and Methods

Technical grade Alachlor with a purity of 97% was procured from M/S Sinochem Private Limited, Mumbai and used in this study. Clinically healthy Black Bengal adult goats (1-1½ years age) weighing between 10-12 kg were used in this experiment. They were caged individually in customized stainless steel metabolic cage. The animals were stall-fed and water was provided *ad libitum*. The composition of feed was 2-part wheat husk, 1-part crushed maize, 1-part crushed gram and 2-part green. The temperature of the animal room was maintained at $22 \pm 3^\circ\text{C}$ and provided with artificial light.

Before starting the experiment, the animals were dewormed once with a mixture of albendazole and rafoxanide (Vetalben-R, Indian Immunologicals) at the dose rate of $7.5\text{ mg kg}^{-1}\text{ b.w.}$. After 21 days of deworming, the animals were acclimatized for 7 days, during which, the body temperature, quality of faeces and urine output were recorded. The Institution Animal Ethics Committee approved the experimental protocol before the study commenced.

Oral Toxicokinetic study of Alachlor

The minimum oral toxic dose was determined using a trial-

and-error method, where six groups of two female goats each received ascending doses of 400, 700, 900, 1000, and 1200 mg/kg b.w. of Alachlor mixed with jaggery, while a control group received only jaggery. Goats receiving 700 mg/kg or higher exhibited severe toxicity within seven days, whereas the 400 mg/kg group showed mild symptoms such as depression, drowsiness, discomfort, frequent urination, and inappetence, which resolved within two hours. The control group showed no toxic signs; thus, 400 mg/kg was established as the minimum oral toxic dose. For the toxicokinetic study, six female goats were divided into two groups: four in the experimental group (Group I) receiving 400 mg/kg b.w. of Alachlor with jaggery, and two in the control group (Group II) receiving only jaggery.

Analysis of toxicokinetic parameters

The experimental data of blood Alachlor against time were fitted to the computerized programme, 'PHARMKIT' supplied by the Deptt. of Pharmacology, JIPMER, Pondicherry, India. The following toxicokinetic parameters were then determined as per slandered formulae (Baagot, 1977 and Notari, 1980) ^[11]:

A, B	:	Zero-time blood Alachlor concentrations intercepts of biphasic intravenous disposition curve. The co-efficient A is the point of intercept of residuals and co-efficient B is based on the terminal elimination phase. These are expressed in $\mu\text{g ml}^{-1}$.
C^0_p	:	The theoretical zero-time blood Alachlor concentration and is expressed as $\mu\text{g ml}^{-1}$ $C^0_p = A + B \mu\text{g ml}^{-1}$
K_a & K	:	The hybrid rate constants of disposition curve. Values of K_a and K are related to the slope of distribution and elimination curve. These rate constants are obtained from the terminal slope of semi-logarithmic plot of blood Alachlor concentrations versus time and are expressed as hr^{-1} (per hour).
$t_{1/2}(k_a)$ & $t_{1/2}(K)$	:	The half-lives of Alachlor in distribution and elimination phase respectively. They are expressed as hr. $t_{1/2} K_a = \frac{0.693}{K_a}$ $t_{1/2} K = \frac{0.693}{K}$
V_{dc}	:	The apparent volume of distribution of Alachlor in the central compartment and expressed as L kg^{-1} . $V_{dc} = \frac{D}{A + B}$ Where, D is the dose (mg kg^{-1})
$V_{d_{area}}$	:	The apparent volume of Alachlor distribution based on total area under blood Alachlor concentration versus time curve (area method) and expressed as L kg^{-1} . $V_{d_{area}} = \frac{D}{AUC \times K}$
AUC	:	The total area under the Blood Alachlor concentration versus time curve from ' T_0 ' to ' t ' after administration. The unit of measurement is $\mu\text{g hr ml}^{-1}$. $AUC = \frac{A}{K_a} + \frac{B}{K}$ (Ritschel, 1980)
Cl_B	:	Total body clearance of a Alachlor representing the sum of all clearance processes in the body and expressed as $\text{L kg}^{-1} \text{ hr}^{-1}$. $Cl_B = V_{d_{area}} \times \beta$
MRT	:	Mean residence time expressed as hr.
$T \sim P$	:	Tissue – blood ratio which is obtained by using the equation; $T \sim P = \frac{K_{12}}{K_{21} - K}$

The toxicological parameters were determined for each animal individually and then mean and standard error (SE) of each parameter was calculated from the sum up values of four animals.

Analysis of Alachlor in blood, urine and tissue

Analysis of Alachlor from blood (plasma), urine and tissue were done.

Analysis of Alachlor from different substrates involves three steps i.e. extraction, clean up and estimation.

Extraction

Plasma: To a sample of whole Blood (2ml), 10% Sodium chloride (10 ml), Magnesium sulphate(3gm) and Ethyl acetate (10ml) was added, shaken by vortex machine for 1min, let the

mixture stand for 30 minutes and shaken for 1 min and then centrifuged for 10,000 RPM for 15 mins and 5 ml of supernatant is taken for analysis.

Urine: 10 ml of urine was taken to a dried reparatory funnel (100ml) and magnesium sulphate (3gm) and Ethyl acetate(10ml) was added to it, shaken vigorously for 1 min. and then allowed to settle for 30 mins. Then the mixture is shaken for 1 min and then centrifuged for 10,000 RPM for 15 mins.

Faeces: To a sample of faeces(1gm),10% NaCl(10ml), Magnesium sulphate(3gm), Ethyl acetate(10ml) was added and shaken for 1 min. let the mixture allowed to settle for 30 mins, then shaken for 1 min and centrifuged for 10000 rpm

for 15 mins and supernatant was taken for analysis

Tissue: Liver, kidney, heart, lung, spleen.

The tissue (1gm) was first minced with scissors and then homogenized in Remi-tissue homogenizer (RQ-127A) for 4 min with Ethyl acetate (10ml) and 10% NaCl(10ml). The homogenate was again dissolved in Ethyl acetate(10ml). This mixture is then centrifuged for 10,000 rpm for 15 mins and supernatant is taken for analysis.

Clean up procedures

Clean up for Tissues: PSA (50mg) and C18 (50mg) and Magnesium sulphate (300mg) were added in the supernatant fraction and shaken for 1 min and then the supernatant fraction is taken and filtered through the 0.2-micron syringe filter and 1 µl is pushed through injector.

Clean up for faeces: PSA (50 mg) and C18 (50mg) and Magnesium sulphate (300mg) were added in the supernatant fraction and shaken for 1 min and then the supernatant fraction is taken and filtered through the 0.2-micron syringe filter and 1 µl is pushed through injector.

Recovery experiment

Alachlor

Recovery percentage of Alachlor from different substrates was carried out to ascertain the reliability of the analytical method after fortifying the different substrates viz., blood, urine, faeces and tissues with technical grade Alachlor at different spike concentration of 0.005, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1(µg/ml). After necessary work up, the concentration of Alachlor from different tissues/substrates was analysed by Gas Chromatography (GC).

Estimation of concentration of Alachlor

The quantitative estimation of Alachlor was performed using a Varian 3800 Gas Chromatography (Varian Inc., USA) equipped with a ⁶³Ni electron capture detector (ECD). High-purity nitrogen was used as both the carrier gas and the make-up gas, with carrier and make-up gas flow rates set at 1 ml/min and 25 ml/min, respectively. The chromatographic separation was achieved using a VF-1ms column (15M × 0.25MM ID, DF = 0.25), and the retention time of Alachlor was determined to be 5.35 minutes. A standard and sample volume of 2 µl were injected using a 10 µl SGE micro syringe. The drug concentration was estimated by comparing the sample peaks with an external standard. For calibration, a stock solution of 10 ppm of analytical-grade Alachlor was prepared in distilled water. The retention time of Alachlor in the standard solution was confirmed to be 5.35 minutes, ensuring accurate identification and quantification. Residue analysis was conducted by injecting 2 µl of both standard and sample solutions, and the concentration was determined by comparing the chromatographic response with the external standard.

The concentration of Alachlor in Blood and tissue was calculated using following equation

$$\text{Concentration of Alachlor in blood } (\mu\text{g/ml}) = \frac{a_2 \times v_2 \times C}{a_1 \times v_1}$$

$$\text{Residue of Alachlor in tissue } (\mu\text{g/gm}) = \frac{a_2 \times v_2 \times C}{a_1 \times g}$$

a_1 = area of standard chromatogram

a_2 = area of sample chromatogram

V_2 = Final volume of sample after processing (ml)

V_1 = Volume of Volume or urine taken for processing (ml)

C = concentration of standard

g = amount of tissue taken for processing.

Statistical analysis

The results were expressed as Mean ± Standard error (S.E.). The data were analysed statistically using general linear model with univariate data in SPSS 10.0 version of software.

Results

Standardization and Recovery Study of Alachlor

Analytical method for estimation of Alachlor

Alachlor was estimated by using a Varian 3800 Gas Chromatography. The Chromatogram (Fig. 1) showed well resolved peak of technical grade alachlor and the retention time ® was at 5.35 min under the operating condition of the chromatograph as stated. Ascending doses of alachlor i.e. 0.005, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1 ppm showed peak areas which is on plotting, the linearity is found. (Table 1 and Fig. 1).

Tissue recovery experiment

The result of *in vitro* recovery experiments of alachlor from blood (Table 2), urine and faeces (Table 3). The recovery percentage from goat's blood, urine, faeces and different tissues fortified with 0.1 ppm of alachlor varied from 90-94% (Table 4). The limit of detection and quantification of alachlor using the above method was 0.02 and 0.06 ppm and accuracy of the assay was 93%. The validation of data has been depicted in Table 6. Therefore, the method was found to be highly satisfactory and subsequently used for extraction, clean up and estimation of alachlor from blood, urine and faeces of goat.

Table 1: Peak area values of known concentration of alachlor in ethyl acetate following estimation by Gas Chromatography (Mean values of 3 replicate)

Concentration (µg/ml)	Average peak are	Peak are (x10 ⁴)
0.005	4145	0.41
0.01	8631.7	0.86
0.025	23476.8	2.3
0.05	48208.5	4.82
0.1	101019.2	10.1
0.25	234635	23.4
0.5	457467	45.7
1	908304	90.8

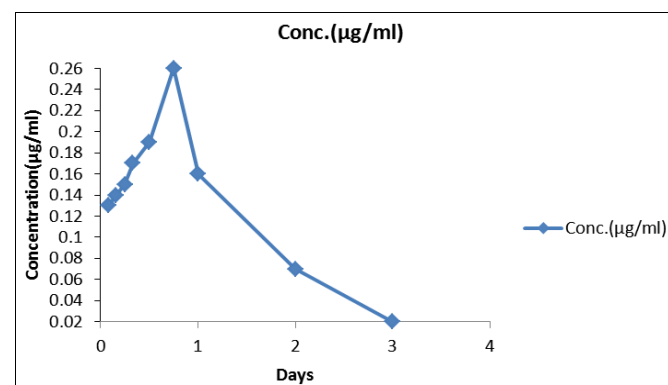


Fig 1: Blood level of Alachlor (µg/ml) following single oral dose administration at 400mg/kg in goats

Table 2: Blood level of Alachlor ($\mu\text{g/ml}$) following single oral dose administration at 400mg/kg in goats (Mean value of four replicates with SE)

Time(hr)	Concentration ($\mu\text{g/ml}$)
0.08	0.13 ± 0.04
0.16	0.14 ± 0.04
0.25	0.15 ± 0.04
0.33	0.17 ± 0.08
0.50	0.19 ± 0.04
0.75	0.26 ± 0.08
1	0.16 ± 0.08
2	0.07 ± 0.03
3	0.02 ± 0.01

Table 3: Concentration of alachlor in urine and faeces following single dose oral administration at 400mg/kg in goats (values are mean with SE, n=4)

Time (hr.)	Concentration (ppm)	
	Urine	Faeces
0 – 24	1.59 ± 0.60	7.59 ± 0.83
24 – 48	0.86 ± 0.26	5.67 ± 0.68
48 – 72	0.12 ± 0.04	2.82 ± 0.24
72 – 96	BDL	1.95 ± 0.46
96 – 120	-	1.08 ± 0.18
12- - 144	-	BDL

Table 4: Recovery of alachlor from different substrates of goats following fortification at 0.10ppm (Mean values of 3 replicate)

Substrates	Mean recovery (%)
Liver	90.0
Kidney	91.0
Heart	92.0
Lung	91.0
Spleen	90.0
Urine	94.0
Faeces	93.0
Blood	92.0

Table 5: Data validation

Accuracy	93 ± 1.68
Slope	90.68
Intercept	0.308
Linearity range	0.01 - $1\mu\text{g/ml}$
Correlation Coefficient	0.99
SE of intercept	0.591350573
LOD	$0.021520257\mu\text{g/ml}$
LOQ	$0.0652129\mu\text{g/\mu l}$

Oral Toxicokinetic study:

Oral administration of alachlor at 400mg kg^{-1} produced minimum toxic symptoms like discomfort, frequent urination and drowsiness, depression. All the symptoms were waned out within 2 hours of administration. Control group of goats which received jaggary did not exhibit any signs of toxicity. The oral LD₅₀ of alachlor in rat was 930-1350 mg kg^{-1} (Rotterdam Convention, 2011) ^[14] and therefore the dose used considered to be non-toxic.

Toxicokinetics and recovery of alachlor following single oral dose administration

Mean values of blood concentration of alachlor at different time intervals after single oral dose administration at 400mg kg^{-1} b.wt. has been depicted in Table 2 and Fig. 1. It is observed from Table 2 that blood level of alachlor detected at 0.08 hr. post dosing (pd) ($0.13 \pm 0.04 \mu\text{g ml}^{-1}$). The concentration then gradually increased and the maximum blood concentration (C^{Bmax} ; $0.26 \pm 0.08 \mu\text{g ml}^{-1}$) was

achieved at 0.75 hr. Therefore, the concentration started to decline bit by bit and minimum blood concentration (C^{Bmin} ; $0.02 \pm 0.01 \mu\text{g ml}^{-1}$) was detected in samples collected at 3 hr. However, alachlor could not be detected in blood sample of goat collected beyond 3 hr. pd.

The mean values of different toxicokinetic parameters have been presented in Table 6. It is observed from Table 6 that the mean absorption rate constant (K_a) was higher ($0.09 \pm 0.01 \text{ min}^{-1}$) indicating fast rate of absorption of alachlor from Gastrointestinal tract of goat. The higher apparent volume of distribution (V_d area) value ($1.76 \pm 0.49 \text{ L Kg}^{-1}$) indicates wide distribution of the compound in all the compartment of the body, as high lipid soluble chemicals are usually distributed in body fluids and tissue. The larger volume of distribution along with lower values of elimination half-life ($t_{1/2K}$ $54.12 \pm 8.94 \text{ mins}$) and shorter value of MRT ($1.10 \pm 0.12 \text{ hr}$) suggest shorter per system in systemic circulation. The higher mean value of CLB ($0.01 \pm 0.002 \text{ L/kg/min}$) and Kel ($0.01 \pm 0.0025 \text{ min}^{-1}$) also substantiate the above observation.

Recovery from urine and faeces

Recovery from urine

The mean concentration with standard error (SE) of alachlor recovered from urine at different time intervals of goats following single high oral dose administration have been described in Table 3 and Fig. 2. The mean quantity of alachlor recovered at 24, 48, 72 hr from urine of goat were 1.59 ± 0.63 , 0.86 ± 0.26 , $0.12 \pm 0.04 \mu\text{g ml}^{-1}$ respectively. However, alachlor could not be recovered from urine after 72 hr. pd. Maximum quantity of alachlor was found to be excreted through urine by 24 hr. pd. considering the dose of alachlor administered orally the amount excreted in urine is very small.

Recovery from faeces

The mean value with standard error of alachlor recovered from faeces at different time interval of goats following single oral high dose administration have been described in Table 3 and Fig. 2. 5.67 ± 0.68 , 2.82 ± 0.24 , 1.95 ± 0.46 , $1.08 \pm 0.18 \mu\text{g ml}^{-1}$ respectively. The maximum quantity was recovered from faeces of goat by 24 hr. pd. From the figure, it is evident that the alachlor is excreted predominantly in faeces. Davidson *et al* (1994) ^[4] reported that alkyl groups on the phenyl portion of the alachlor causes biliary elimination to be favoured over urinary elimination.

Discussion

The present study aimed to standardize an analytical method for alachlor estimation and investigate its toxicokinetic following single oral administration in goats. The validated gas chromatography method provided reliable quantification of alachlor, demonstrating well-resolved chromatographic peaks with a retention time of 5.35 minutes. The recovery study revealed a high accuracy (93%) with a recovery percentage ranging from 90% to 94% across different biological matrices, indicating the method's efficiency in detecting alachlor residues. These findings align with previous studies that highlight gas chromatography as an effective tool for pesticide residue analysis (Zhao *et al.*, 2017; Kumar *et al.*, 2020) ^[9, 19].

The toxicokinetic study demonstrated that orally administered alachlor (400 mg/kg body weight) was rapidly absorbed, with a detectable blood concentration appearing at 0.08 hours post-dosing and reaching a peak concentration ($C_{\text{max}} = 0.26 \pm 0.08 \mu\text{g/mL}$) within 0.75 hours. The elimination of alachlor from the bloodstream was rapid, with a short elimination half-

life ($t_{1/2} = 54.12 \pm 8.94$ minutes) and a mean residence time (MRT) of 1.10 ± 0.12 hours, suggesting minimal persistence in systemic circulation. The high volume of distribution ($V_d = 1.76 \pm 0.49$ L/kg) indicated that alachlor was widely distributed in body tissues, likely due to its lipophilic nature (Smith & Jones, 2015) ^[16]. The higher clearance rate ($CL_B = 0.01 \pm 0.002$ L/kg/min) and elimination rate constant ($K_{el} = 0.01 \pm 0.0025$ min⁻¹) further confirmed its rapid metabolism and excretion.

The excretion profile indicated that alachlor was predominantly eliminated through faeces rather than urine. The maximum recovery from faeces occurred within 24 hours post-dosing, with detectable levels up to 72 hours. Urinary excretion was relatively minor, with the highest concentration detected at 24 hours post-dosing. This observation aligns with Davidson *et al.* (1994) ^[4], who reported that alachlor undergoes biliary elimination due to the presence of alkyl groups on its phenyl moiety. The predominance of faecal excretion suggests hepatic metabolism followed by biliary excretion as the primary route of elimination. Other studies have also reported similar excretion pathways for organochlorine pesticides in ruminants (García *et al.*, 2019) ^[7].

Toxicity symptoms observed following oral administration were mild and transient, including discomfort, frequent urination, drowsiness, and depression, all of which resolved within two hours. Given that the oral LD₅₀ of alachlor in rats is 930-1350 mg/kg (Rotterdam Convention, 2011) ^[14], the administered dose (400 mg/kg) in goats was considered non-toxic. This is consistent with earlier studies showing that alachlor exhibits species-specific toxicity variations (Patil *et al.*, 2016) ^[12].

Conclusion

The study successfully validated an analytical method for alachlor detection with high recovery efficiency. The toxicokinetic evaluation revealed rapid absorption, widespread distribution, and efficient elimination primarily via faeces. The findings suggest that alachlor undergoes rapid metabolism and excretion, minimizing systemic persistence. The transient nature of observed toxicity symptoms at the administered dose further supports its low acute toxicity in goats. These results provide crucial insights into the pharmacokinetic behavior of alachlor, contributing to risk assessment and environmental safety considerations in livestock management. Further studies focusing on chronic exposure and residue accumulation in edible tissues are recommended for a comprehensive safety evaluation.

Conflict of Interest

Not available

Financial Support

Not available

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How to Cite This Article

Dasgupta S, Mandal TK, Singh E, Sinha BP, Pandey AK, Sar TK, *et al.* Toxicokinetic evaluation of alachlor in goats: Elimination kinetics, tissue distribution and recovery analysis using gas chromatography. *International Journal of Veterinary Sciences and Animal Husbandry*. 2026;SP-11(4):248-252.

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