https://doi.org/10.52973/rcfcv-e35706 Revista Científica, FCV-LUZ / Vol. XXXV

Lead toxicokinetics following intravenous and oral administration in non–lactating ewe: A preliminary study

Toxicocinética del plomo tras administración intravenosa y oral en ovejas no lactantes: Un estudio preliminar

Boufedda Nadia*, *Sellaoui Sassia**, *Arab Hadda*, *Boudaoud Amine*, *Mehennaoui Smail

*University of Batna 1, Institute of Veterinary and Agricultural Sciences, Veterinary Department, Research laboratory environment, production, and animal health (LESPA). Batna 05000, Algeria. *Corresponding author: sacia.sellaoui@univ–batna.dz*

ABSTRACT

The present study aims to describe the metabolism of the lead in sheep (*Ovis aries*) by implementing a toxicokinetic approach and to determine the bioavailability. A clinically healthy, one–year–old, non–lactating ewe (40 kg) received a single intravenous dose of lead acetate (0.165 mg Pb·kg^{-1}) followed by oral administration (2.5 mg Pb·kg^{-1}) after a 40 day washout. Lead, zinc, copper and calcium levels in the diet were measured before feeding. Serial blood samples were collected over 5 hours (h) (intravenous) and 9 h (oral) and analyzed by electrothermal atomic absorption spectrophotometry. Concentration–time data were fitted to a two compartment model (bicompartmental biexponential for intravenous; biexponential with absorption term for oral) to derive distribution and elimination halflives, clearance, volumes of distribution, mean residence time, area under the curve, and absolute bioavailability. Analysis of ewe feed revealed excessive calcium intake. Following intravenous dosing, lead peaked at 870 µg·L^{-1}, with a rapid distribution ($T_{½}\alpha$ = 0.004 h) and slow elimination ($T_{½}\beta$ = 6.4 h). Oral administration yielded a lower peak (522 µg·L^{-1}) with absolute bioavailability of only 2% (High dietary calcium likely suppressed gastrointestinal absorption), while steady–state volume of distribution (0.275 L·kg^{-1}) indicated extensive tissue accumulation. The findings highlight compartmental modelling as a critical tool for assessing lead toxicokinetic in ruminants.

Key words: Lead; ewe; toxicokinetics; compartment analysis; bioavailability

RESUMEN

El propósito de este estudio es describir el metabolismo del plomo en ovejas (*Ovis aries*) con un enfoque toxicocinético y determinar su biodisponibilidad. Se utilizó una oveja clínicamente sana de un año de edad y no lactante (40 kg) quien recibió una dosis única de acetato de plomo intravenosa (0,165 mg Pb·kg^{-1}), seguida de una administración oral (2,5 mg Pb·kg^{-1}) después de un periodo de depuración de 40 días. En el presente estudio, se midieron los niveles de plomo, zinc, cobre y calcio presentes en la dieta antes de su administración. Se colectaron muestras sanguíneas en serie durante un período de cinco horas (intravenosa) y nueve horas (oral) y se analizaron mediante espectrofotometría de absorción atómica electrotérmica. Tambien se ajustaron los datos de concentración–tiempo a un modelo bicompartimental (bi–exponencial para intravenosa; bi–exponencial con término de absorción para oral) para calcular las vida mediade distribución y eliminación, el aclaramiento, los volúmenes de distribución, el tiempo medio de residencia, el área bajo la curva y la biodisponibilidad absoluta. El análisis del alimento mostró un exceso de calcio dietético. Tras la administración intravenosa, la concentración máxima de plomo alcanzó un pico de 870 µg·L^{-1}, una distribución rápida ($T_{½}\alpha$ = 0,004 h) y una eliminación lenta ($T_{½}\beta$ = 6,4 h). La administración oral del compuesto resultó en un pico significativamente más bajo (522 µg·L^{-1}) con una biodisponibilidad absoluta de 2% probablemente atribuido a la interferencia del calcio dietético en el proceso de absorción gastrointestinal del compuesto. El volumen de distribución en estado estacionario (0,275 L·kg^{-1}) indicó una acumulación tisular extensa. Los resultados ponen de manifiesto la relevancia del modelado compartimental como una herramienta fundamental para la evaluación de la toxicocinética del plomo en rumiantes.

Palabras clave: Plomo; oveja; toxicocinética; análisis compartimental; biodisponibilidad

Received: 24/05/2025 Accepted: 06/10/2025 Published: 30/10/2025

INTRODUCTION

Lead (Pb) is the most persistent ubiquitous, highly toxic heavy metal with no known biological role. Due to its non–biodegradable nature and widespread use, it accumulates in the environment with increasing hazards. Ruminants are highly susceptible to Pb toxicity and its wide range of adverse effects [1, 2, 3]. Due to their non–discriminatory eating habits, cattle (*Bos taurus*) are more frequently affected compared to other species [4].

This toxicity manifests as nervous and digestive symptoms, including anorexia, blindness, convulsions, and opisthotonos. Additionally, cattle often suffer from subclinical Pb poisoning due to grazing on Pb–contaminated pastures [5, 6, 7, 8]. Sheep (*Ovis aries*) living in contaminated areas are no less sensitive to Pb toxicity [3, 9, 10, 11]. The primary indicators of chronic Pb exposure in ruminants include blood Pb levels [8, 11, 12], zinc protoporphyrin [13], and δ–aminolevulinic acid dehydratase (δ–ALAD) inhibition [14] all of which reflect the presence of metabolically active Pb in the body.

Unlike in humans, where several studies have permitted to propose some pharmacokinetic models allowing a better characterization of the Pb disposition in the human's organism [15, 16, 17], in ruminants' studies are still lacking, and many aspects need further clarification.

Among the few studies conducted on the subject, one notable example is the work by Milhaud and Mehennaoui [13] who applied a two–compartment pharmacokinetic model to monitor blood Pb levels in cattle during a chronic Pb exposure. To study the transfer of Pb into milk, muscle and offals in the lactating ewes, Mehannaoui *et al.* [18] employed a toxicokinetic approach and Waldner *et al.* [19] used a single–component exponential model to describe the changes in blood lead levels over time in exposed cattle after ingestion of abandoned batteries.

Given that understanding the metabolic balance of a metal is more effectively achieved through a kinetic approach than by simply measuring the amounts ingested and excreted in urine and feces [20] and considering the limited research on Pb toxicokinetics in ruminants, a preliminary study was carried out to investigate the disposition of Pb in sheep following different routes of exposure. A single dose of Pb acetate was administered intravenously (IV) and orally. Toxicokinetic parameters were determined for each of the two routes of administration with a focus on the absolute bioavailability of Pb. The absolute bioavailability of Pb refers to the fraction of Pb that reaches the systemic circulation after oral ingestion and absorption from the gut [11, 20, 21]. The study aimed at describing Pb fate within the body in sheep using a toxicokinetic approach.

MATERIALS AND METHODS

This work was conducted at the sheepfold of the Veterinary Department at the University of Batna 1, after approbation of the experimental protocol by the Scientific Committee of the Institute of Veterinary and Agricultural Sciences.

For the experiment, a clinically healthy, non–lactating one–year–old ewe (40 kg), was recruited. Body weight was measured using a mechanical scale designed for sheep and calves (PATURA KG; Ref. 430350; Germany). Before and during the experiment, water, commercial feed and dry litter were available. The levels of Pb, zinc (Zn), copper (Cu), and calcium (Ca) were measured in the feed before it was fed to the ewe. Animal welfare and access to veterinary care have been insured throughout the period of the experiment.

Experimental design

The ewe received a single IV bolus administration of Pb acetate at a dose of 0.165 mg Pb·kg^{-1} body weight. The total dose administered (6.6 mg) was diluted in 2 mL of sterile water for injection to achieve a concentration of 3.3 mg Pb·mL^{-1}. Blood samples (4 mL) were collected from the left jugular vein into heparinized tubes under vacuum, which is known not to interfere with the analysis of trace elements, metals or metalloids. Sampling was performed at successive time points: 2, 5, 10, 15, 30 min and 1 hour (h), 1h 30 min, 2 h, 3 h, 4 h, 5h after dosing.

Additionally, after a 40 day (d) washout period, the ewe received a single oral administration of Pb acetate at a dose of 2.5 mg Pb·kg^{-1} of body weight. Pb was encapsulated in a gelatin capsule and placed over the base of the tongue to ensure that the animal swallowed the capsule. Serial blood sampling was performed at different consecutive time points: 0, 0.25, 0.5, 1, 1.5, 2.5, 4, 5, 6 and 9 h.

Chemical analysis

Determination of blood Pb concentrations

A volume of 1 mL of blood was transferred to a teflon bottle and 5 mL of HNO_3 was added. The sample was left at room temperature for at least 30 min. The teflon bottle without its lid, was placed on a sand bath (Combiplac – Sand; J.P.Selecta; Spain) and heated at 150°C for one hour until the acid volume was reduced to 1 mL. Subsequently, 2 mL of HNO_3 and 1 mL of concentrated HCl were added to the sample. The bottle was sealed and heated on a sand bath at 150°C for one h. The digested sample was then transferred to a 50 mL vial, filtered and made up to final volume with distilled water. Pb concentrations in whole blood were measured by electrothermal atomic absorption spectrophotometry (Analyst 100; PerkinElmer; USA). The operating conditions were drying at 200°C, ashing at 700°C and atomizing at 1800°C. All samples were analyzed in duplicate. The linearity of the calibration curve extended to 50 µg·L^{-1}. The detection limit was estimated at 4 µg·L^{-1}.

Determination of feed trace element concentrations

Hay and granulated feed samples were collected from the ewe's diet to assess the intake of trace elements. The feed samples were subjected to a wet digestion using two pure acids, HNO_3 and $HClO_4$ [22]. First, 5 mL of HNO_3 (15N) was added to 1 g of sample and boiled on a sand bath for 30 min. Then, 3 mL of 70% $HClO_4$ was added, and the mixture was boiled until the acidic volume was reduced to 1 mL. After cooling to ambient temperature, 10 mL of deionized water was added and filtered through a Watmann filter (N 540) in a volumetric flask and adjusted to the final volume of 50 mL. Pb and Cd concentrations were determined by using graphite furnace atomic absorption spectrometry (Analyst 100; PerkinElmer; USA), while Ca, Zn, Fe and Cu were measured by flame atomic absorption spectrometry (Shimadzu AA-6800; Japan).

Toxicokinetics analysis

Blood Pb concentrations following IV and oral administrations were subjected to compartmental analysis, non–linear least square regression, using a program adapted from PK Solver software [23]. A biexponential equation, representing a bicompartimental model with elimination occurring from the central compartment, was fitted to the blood Pb concentrations for IV and oral administrations:

For IV administration:

- $C(t) = Ae^{-\alpha t} + Be^{-\beta t}$

For oral administration:

- $C(t) = Ae^{-\alpha t} + Be^{-\beta t} - Ce^{-Kat}$

Where: $C(t)$ is the blood concentrations at time t, α and β are the exponential terms, A is the blood lead concentration at time t=0 during the distribution phase, B is the blood lead concentration at time t=0 during the elimination phase and Ka is the absorption constant.

The area under the blood lead concentration curve (AUC) can either be calculated by the trapezoidal method or estimated as follows:

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta}$$

The half–life ($T_{½}\alpha$) was determined during the distribution phase using the following equation:

$$T_{1/2}\alpha = \frac{\ln 2}{\alpha} = \frac{0.693}{\alpha}$$

The half–life ($T_{1/2}\beta$) was determined during the elimination phase as below:

$$T_{1/2}\beta = \frac{\ln 2}{\beta} = \frac{0.693}{\beta}$$

The total body clearance (Cl) was calculated as following:

$$Cl = {Dose\ IV}/{AUC_{\infty}^{0}}$$

Following IV bolus administration of a compound, two distinct volumes of distribution can be determined [24].

» The volume of distribution of the central compartment Vc:

$Vc = V \times \frac{Dose_{IV}}{C_0}$ where C_0 is the concentration at t=0

» The volume of distribution at steady state V_{ss}:

$V_{ss} = Cl \times MRT_{(IV)}$

The Mean Residence Time (MRT) after IV administration was calculated as follows:

$$MRT = \frac{AUMC_{0-\infty}}{AUC_{0-\infty}}$$

Where $AUMC_{0-\infty}$ is the area under the moment curve and $AUC_{0-\infty}$ is the area under the blood concentration curve. In addition, the ratio of the area under the moment curve to the area under the concentration–time curve (AUMC/AUC), commonly used as a definition for the MRT of drug molecules in the body should rather be considered as a method of evaluating this parameter.

The Absolute bioavailability F is the fraction absorbed via gastro–intestinal route and was calculated as follows:

$$F = \frac{AUC_{\infty}^{0}\ per\ os}{AUC_{\infty}^{0}\ IV} \times \frac{Dose\ IV}{Dose\ per\ os}$$

RESULTS AND DISCUSSION

The bioavailability of Pb has been assessed in mice (*Mus musculus*), monkeys (*Platyrrhini* sp.), rabbits (*Oryctolagus cuniculus*), rats (*Rattus norvegicus*), and swine (*Sus scofa domesticus*) [25, 26, 27] but to our knowledge, no IV Pb dosing data have existed for ruminants prior to this study. This is the first investigation to administer Pb intravenously in ewes, providing novel insights into Pb toxicokinetics in sheep using both IV and oral administration of Pb acetate.

The use of a non–lactating ewe allowed us to avoid potential confounding factors related to lactation on Pb kinetics [19, 28, 29]. Similarly, the oral dose of 2.5 mg·kg^{-1} was selected to prevent clinical toxicity while ensuring measurable blood Pb levels for kinetic analysis. No clinical signs of Pb intoxication were observed throughout the study, confirming the safety of the selected dose for toxicokinetic purposes. According to Rodrigues–Estival *et al.* [14], sheep show clinical poisoning at blood Pb ≈350 µg·L^{-1}, while Mehennaoui *et al.* [5] attest that this requires more than twice this concentration (750 g·L^{-1}), and Sellaoui *et al.* [12] observed anemia at 445 µg·L^{-1} after chronic dosing. In this study, postdosing levels far exceeded these thresholds without any signs of toxicity. The bioavailability of heavy metals depends largely on their oxidation state and solubility [29, 30]. Regarding the chemical species of the lead, the Pb acetate we used is a reference soluble compound that is expected to fully dissolve in gastrointestinal fluids upon ingestion [31].

Dietary intakes of Cd and Pb proved negligible, as both metals remained below detection limits in the food analysis, thus contributing minimally to systemic Pb levels (TABLE I). Consequently, blood Pb concentrations remained below the detectable threshold until IV dosing. Immediately after IV administration, blood Pb levels rose immediately to a pic of 870 µg·L^{-1} (FIG. 1), followed by rapid decline between 10–30 min (distribution phase) and slower elimination thereafter. Concentrations measured 317 µg·L^{-1} at 5 h post–dosing, demonstrating prolonged circulation despite initial distribution kinetics

TABLE I
Mineral and heavy metal composition of the diet and estimated daily intake in the Ewe

Parameters	Rations			
	Hay	Maize	Barely	Feed intake·day^{-1}
Cu (mg·kg^{-1})		7.8	8	8.4 mg
Zn (mg·kg^{-1})	15	24	19.5	24 mg
Ca (g·kg^{-1})	22	4.7	5.7	24 g
Fe (g·kg^{-1})	106.5	70	87	141.9 g
Cd (µg·kg^{-1})	< DL	< DL	< DL	< DL
Pb (µg·kg^{-1})	< DL	< DL	< DL	< DL

DL: Detection Limit

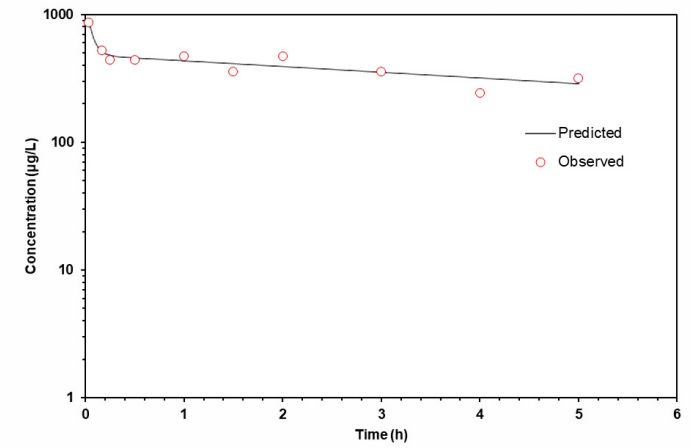

FIGURE 1. Lead blood concentrations in ewe after a single IV administration of 0.25 mg Pb·kg^{-1} body weight

FIGURE 2 illustrates the time course of Pb concentrations following oral administration. Blood Pb levels started to increase from the initial 15 min, reaching a maximum of 522 µg·L^{-1} (predicted value) after 0.54 h. Then the blood Pb concentration initiated a fast decay following a biexponential model. The Pb level in the blood was only 56 µg·L^{-1} 6 hours after oral administration.

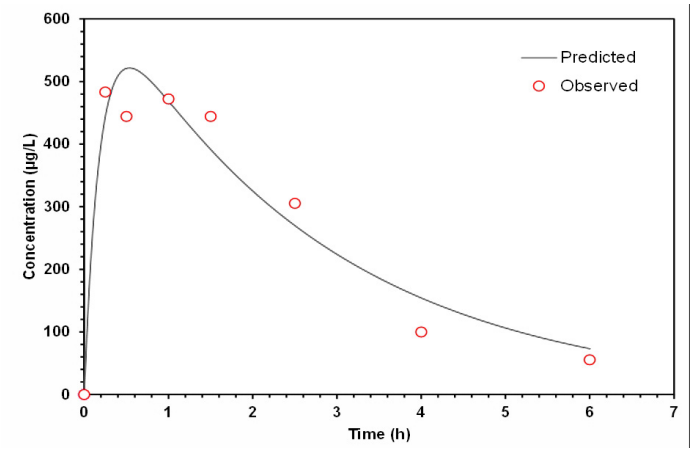

FIGURE 2. Lead blood concentrations in ewe after a single oral administration of 2.5 mg Pb·kg^{-1} body weight

The oral bioavailability of Pb acetate was approximately 2%, consistent with previous reports in ruminants [32], despite differences in the chemical form of Pb and measurement methodologies. This limited absorption can be attributed to multiple physiological and dietary factors. First, rumen microorganisms convert soluble Pb salts into insoluble Pb sulfide, thereby reducing the fraction of absorbable Pb^{2+} [33]. Second, the ewe in this study received a diet particularly rich in calcium (24 g instead of 15 g required by the highest–producing lactating ewe), a well–documented inhibitor of Pb absorption due to its competition for intestinal transporters [17]. High dietary Cahas been shown to significantly reduce Pb uptake [34], whereas Ca deficiency increases systemic Pb bioavailability by enhancing intestinal absorption and decreasing fecal excretion [11, 21, 35].

Together, these ruminal and dietary mechanisms likely explain the low bioavailability observed in this study. As a result, most of the ingested Pb remains unabsorbed and is eliminated via feces [10, 36]. Overall, ruminants show greater tolerance to Pb compared to monogastrics, whose acidic stomach environment increases metal solubility and absorption [37]. The ruminal microbial conversion of soluble Pb to insoluble sulfide is a key physiological mechanism limiting Pb bioavailability in ruminants, although the full adaptation mechanisms to chronic exposure remain unclear.

Pb bioavailability in this study (2%) was lower than approximately 4% reported in lambs [36, 38], consistent with age–related differences in Pb absorption, with younger individuals absorbing substantially more Pb than adults [39] despite their ability to avoid Pb–contaminated forage as adults. Indeed, lambs have an: i) immature rumen, which limits the microbial conversion of soluble Pb into insoluble sulfide [10]; ii) increased intestinal permeability and higher expression of metal transporters (DMT1) [30]; iii) elevated calcium requirements that enhance Pb–Ca competition at the intestinal level [11]; and iv) slower renal clearance, which prolongs systemic exposure [38, 39].

Following IV administration, blood Pb concentrations exhibited a biexponential decline. The same pattern was observed after oral administration, notably during the decay of the blood Pb phase. This biexponential model aligns with previous studies on Pb disposition in cattle, sheep [5, 18, 30, 40], and humans [15, 16, 17], where blood Pb levels after long–term exposure, reflect equilibrium with tissue stores, especially bone. However, a single oral dose in this study did not achieve steady–state blood Pb levels seen in chronic exposure, indicating shorter distribution and elimination phases in single–dose kinetics.

The toxicokinetic parameters for both routes of administration are listed in TABLE II.

Clearance (Cl) values differed markedly between routes: 1.4 L·h^{-1} for IV and 0.12 L·h^{-1} for oral administration. The higher clearance following IV dosing suggests efficient systemic elimination, predominantly via renal pathways, consistent with the kidney's role as a primary organ for Pb excretion [35]. The lower clearance observed after oral administration likely results from limited bioavailability and first–pass effects.

The two–compartment model parameters revealed an extremely short distribution half–life ($T_{1/2}\alpha$) after IV administration (0.004 h), indicating rapid equilibration between blood and peripheral tissues. Oral administration showed a longer distribution half–life (0.3 h), consistent with slower systemic uptake. The elimination half–life ($T_{1/2}\beta$) was 6.4 h post–IV and 2 h post–oral,reflecting slower clearance after IV dosing, possibly due to tissue sequestration and delayed release.These findings contrast with longer elimination half–lives reported in lactating cows after IV Pb acetate [41], suggesting species and physiological status differences.

The absorption half–life ($T_{1/2}Ka$) after oral administration was 0.13 h, indicating rapid gastrointestinal absorption of the fraction of Pb that is bioavailable, despite the overall low systemic availability

TABLE II
Toxicokinetic parameters describing lead disposition in ewe after IV and oral administrations

Parameters	Units	IV administration	Oral administration
A	µg·L-1	153716	1×10^{-6}
α	h	180	2.3
B	µg·L-1	487	685
β	h	0.11	0.37
$T_{1/2}\alpha$	h	0.004	0.3
$T_{1/2}\beta$	h	6.4	2
$T_{1/2}Ka$	h	–	0.13
V	$L\cdot kg^{-1}$	0.05	0.32
Cl	$L\cdot h^{-1}$	1.4	0.12
$AUC_{0-\infty}$	$\mu g\cdot L\text{-}1\cdot h^{-1}$	5342	1709
$AUMC$	$\mu g\cdot L\text{-}1\cdot h^{2}$	41356	4905
MRT	h	7.7	3
V_{ss}	L	11	–
F	%	–	2

Note: A and B are concentrations at t=0 during distribution and elimination phases respectively. $T_{1/2}\alpha$: distribution half–life, $T_{1/2}\beta$: elimination half–life, $T_{1/2}Ka$: constant of absorption half–life, V: central compartment volume, V_{ss}: steady–state volume of distribution, Cl: total blood clearance, AUC: Area Under the Curve, $AUMC$: Area Under the Moment Curve, MRT: mean residence time, F: absolute bioavailability. Pharmacokinetic parameters showed a volume of distribution at steady state (V_{ss}) of 0.275 $L\cdot kg^{-1}$, indicating limited tissue accumulation. Interestingly, the apparent volume of distribution (V) was higher after oral (0.32 $L\cdot kg^{-1}$) than IV administration (0.05 $L\cdot kg^{-1}$), likely due to differences in absorption and early distribution

(F = 2%). This low bioavailability is corroborated by the area under the concentration–time curve ($AUC_{0-\infty}$), which was markedly lower after oral administration (1,709 $\mu g\cdot h^{-1}\cdot L^{-1}$ vs 5,342 $\mu g\cdot h^{-1}\cdot L^{-1}$ IV), confirming minimal systemic uptake. According to Phillips *et al.* [36] and Kumar *et al.* [27], inorganic Pb is not typically absorbed by rumen microorganisms and is effectively excluded from cells.

MRT values were consistent with these observations: 7.7 h after IV administration and 3 h after oral dosing, indicating prolonged systemic exposure when lead bypasses gastrointestinal barriers.

The multiphasic elimination pattern observed, with distinct distribution and elimination phases, supports the involvement of multiple compartments exhibiting different retention and release kinetics [29, 35, 40]. Lead's affinity for soft tissues and bone likely contributes to this kinetic complexity, as these compartments serve as reservoirs that slowly release lead back into circulation.

CONCLUSION

This study provides the first comprehensive assessment of intravenous Pb toxicokinetic in ewes, offering valuable reference data for ruminants. The results confirmed a very low oral bioavailability of Pb acetate (2%) in adult non–lactating ewes, attributed to physiological mechanisms such as ruminal microbial conversion and high dietary calcium intake, both of which significantly limit intestinal absorption.

The biexponential kinetic profiles observed after both IV and oral administration indicate multiphasic disposition with limited tissue distribution and prolonged systemic persistence. The higher systemic clearance and longer elimination half–life after IV dosing reflect tissue sequestration and slow redistribution, underscoring the complexity of Pbkinetics in ruminants. These findings highlight species–specific differences in Pb disposition and the influence of age, diet, and administration route. Future studies should explore chronic exposure scenarios, the role of bone as a long–term reservoir, and the modulation of bioavailability under varying dietary and physiological conditions to better assess health risks and guide regulatory thresholds in livestock.

Conflict of interest

There is no conflict of interest between the authors.

BIBLIOGRAPHIC REFERENCES

[1] Smith KM, Abrahams PW, Dagleish MP, Steigmajer J. The intake of lead and associated metals by sheep grazing mining–contaminated floodplain pastures in mid–Wales, UK: I. Soil ingestion, soil–metal partitioning and potential availability to pasture herbage and livestock. Sci. Total Environ. [Internet]. 2009; 407(12):3731–3739. doi: https://doi.org/d2dgd3

[2] Cowan V Blakley B. Acute lead poisoning in western Canadian cattle — A 16–year retrospective study of diagnostic case records. Can. Vet. J. [Internet]. 2016 [cited Mar 12, 2025]; 57(4):421–426. Available in: https://goo.su/UFCdQO

[3] Darwish WS, Gad TM, Imam TS. Lead, Cadmium and Mercury Residues in the edible offal of sheep and goat with a special reference to their public health implications. Int. J. Adv. Res. [Internet]. 2017; 5(4):189–199. doi: https://doi.org/p89t

[4] Bates N, Payne J. Lead poisoning in cattle. Livestock [Internet]. 2017; 22(4):192–197. doi: https://doi.org/p89w

[5] Mehennaoui S, Charles E, Joseph–Enriquez B, Clauw M, Milhaud GE. Indicators of lead, zinc and cadmium exposure in cattle: II. Controlled feeding and recovery. Vet. Hum. Toxicol. [Internet]. 1988 [cited Mar 25, 2025]; 30(6):550–555. Available in: https://goo.su/KbqWg

[6] Pareja–Carrera J, Mateo R, Rodrigues–Estival J. Lead (Pb) in sheep exposed to mining pollution: implications for animal and human health. Ecotoxicol. Environ. Saf. [Internet]. 2014; 108:210–216. doi: https://doi.org/f6kk8t

[7] Orisakwe OE, Oladipo OO, Ajaezi GC, Udowelle NA. Horizontal and vertical distribution of heavy metals in farm produce and livestock around lead–contaminated goldmine in Dareta and Abare, Zamfara state, Northern Nigeria. J. Environ. Health. [Internet]. 2017; 2017:3506949. doi: https://doi.org/p89x

[8] Kovacik A, Arvay J, Tusimova E, Harangozo L, Tvrda E, Zbynovska K, Cupka P, Andrascikova S, Tomas J, Massanyi P. Seasonal variations in the blood concentration of selected heavy metals in sheep and their effects on the biochemical and hematological parameters. Chemosphere [Internet]. 2017; 168:365–371. doi: https://doi.org/gwghfx

[9] Johnsen IV, Aaneby J. Soil intake in ruminants grazing on heavy metal contaminated shooting ranges. Sci. Total Environ. [Internet]. 2019; 687:41–49. doi: https://doi.org/gnh4nw

[10] Payne J. Livesey C. High lead soils: a potential risk to animal and public health. In: Centre for radiation, chemical and environmental hazards. Chemical Hazards Poisons Report. London (UK): Health Protection Agency. [Internet]. 2010 [cited Apr 21, 2025]; 17:42–45. Available in https://goo.su/28MqwD

[11] Pareja–Carrera J, Martinez–Haro M, Mateo R, Rodríguez–Estival J. Effect of mineral supplementation on lead bioavailability and toxicity biomarkers in sheep exposed to mining pollution. Environ. Res. [Internet]. 2021; 196:110364. doi: https://doi.org/gzrq5w

[12] Sellaoui S, Boufedda N, Boudaoud A, Enriquez B, Mehennaoui S. Effects of repeated oral administration of lead combined with cadmium in non–lactating ewes. Pak. Vet J. [Internet]. 2016 [cited Nov 13, 2024]; 36(4):440–444. Available in: https://goo.su/jOsqs

[13] Milhaud G, Mehennaoui S. Indicators of lead, zinc and cadmium exposure in cattle: 1. Results in a polluted area. Vet. Hum. Toxicol. [Internet]. 1988 [cited 02 Dec, 2023]; 30(6):513–517. Available in: https://goo.su/zTsYz3

[14] Rodrígues–Estival J, Barasona JA, Mateo R. Blood Pb and δ–ALAD inhibition in cattle and sheep from a Pb–polluted mining area. Environ. Pollut. [Internet]. 2012; 160:118–124. doi: https://doi.org/cv6bsw

[15] Leggett RW. An Age–specific Kinetic Model of Lead Metabolism in Humans. Environ. Health Perspect. [Internet]. 1993; 101(7) 598–616. doi: https://doi.org/cgsvpb

[16] National Research Council (NRC). Potential health risks to DOD firing–range personnel from recurrent lead exposure [Internet]. Washington DC (USA): The National Academies Press; 2013 [cited 08 Apr 25]; 198 p. doi: https://doi.org/p9bh

[17] Rădulescu A, Lundgren S. A pharmacokinetic model of lead absorption and calcium competitive dynamics. Sci. Rep. [Internet]. 2019; 9:14225. doi: https://doi.org/p9bm

[18] Mehennaoui S, Houpert P, Federspiel B, Joseph–Enriquez B, Kolf–Clauw M, Milhaud G. Toxicokinetics of lead in the lactating ewe: variations induced by cadmium and zinc. Environ. Sci. [Internet]. 1997 [cited Jun 27, 2023]; 5(2):65–78. Available in: https://goo.su/8UjoBs

[19] Waldner C, Checkley S, Blakley B, Pollock C, Mitchell B. Managing lead exposure and toxicity in cow–calf herds to minimize the potential for food residues. J. Vet. Diagn. Investig. [Internet]. 2002; 14(6):481–486. doi: https://doi.org/d73dx5

[20] Casteel SW, Weiss C, Henningsen GM, Brattin WJ. Estimation of relative bioavailability of lead in soil and soil–like materials using young swine. Environ. Health Perspect. [Internet]. 2006; 114(8):1162–1171. doi: https://doi.org/b3cffq

[21] Beyer WN, Basta NT, Chaney RL, Henry PFP, Mosby DE, Rattner BA, Scheckel KG, Sprague DT, Weber JS. Bioavailability tests accurately estimate bioavailability of lead to quail. Environ. Toxicol. Chem. [Internet]. 2016; 35(9):2311–2319. doi: https://doi.org/f8zz9t

[22] Uddin AH, Khalid RS, Alaama M, Abdualkader AM, Kasmuri A, Abbas SA. Comparative study of three digestion methods for elemental analysis in traditional medicine products using atomic absorption spectrometry. J. Anal. Sci. Technol. [Internet]. 2016; 7:6. doi: https://doi.org/gf3tj3

[23] Zhang Y, Huo M, Zhou J, Xie S. PK Solver: an add program for pharmacokinetic and pharmacodynamics data analysis in Microsoft Excel. Comput. Methods Programs Biomed. [Internet]. 2010; 99(3):306–314. doi: https://doi.org/cpg7rf

[24] Fan J, de Lannoy IAM. Pharmacokinetics. Biochem. Pharmacol. [Internet]. 2014; 87(1):93–120. doi: https://doi.org/ggjs9w

[25] Aungst BJ, Dolce JA, Fung HL. The effect of dose on the disposition of lead in rats after intravenous and oral administration. Toxicol. Appl. Pharmacol. [Internet]. 1981; 61:48–57. doi: https://doi.org/c69j8w

[26] Palminger–Hallén I, Jönsson S, Karlsson MO, Oskarsso A. Toxicokinetics of lead in lactating and nonlactating mice. Toxicol. Appl. Pharmacol. [Internet]. 1996; 136(2):342–347. doi: https://doi.org/bk7pt2

[27] Kumar A, Kumar A, Cabral–Pinto MMS, Chaturvedi AK, Shabnam AA, Subrahmanyam G, Mondal R, Kumar–Gupta D, Malyan SK, Kumar SS, A Khan S, Yadav KK. Lead toxicity: Health hazards, influence on food chain, and sustainable remediation approaches. Int. J. Environ. Res. Public Health. [Internet]. 2020; 17(7): 2179. doi: https://doi.org/gjqqg3

[28] Maldonado–Vega M, Cerbón–Solorzano J, Albores–Medina A, Hernández–Luna C, Calderón–Salinas JV. Lead: intestinal absorption and bone mobilization during lactation. Hum. Exp. Toxicol. [Internet]. 1996; 15(11):872–877. doi: https://doi.org/cspkbn

[29] Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for lead. US Department of Health and Human Services, Public Health Service. [Internet]. 2020; 583 p. doi: https://doi.org/gnt95d

[30] Mitra P, Sharma S, Purohit P, Sharma P. Clinical and molecular aspects of lead toxicity: An update. Crit. Rev. Clin. Lab. Sci. [Internet]. 2017; 54(7–8):506–528. doi: https://doi.org/gjsdk9

[31] Oomen AG, Tolls J, Sips A, Van den Hoop MA. Lead speciation in artificial human digestive fluid. Arch. Environ. Contam. Toxicol. [Internet]. 2003; 44:107–115. doi: https://doi.org/bqw334

[32] Oehme FW. Toxicity of heavy metals in the environment: Part I. New York, USA: Marcel Dekker Inc; 1978.

[33] Wu H, Meng Q, Zhou Z, Yu Z. Ferric citrate, nitrate, saponin and their combinations affect *in vitro* ruminal fermentation, production of sulphide and methane and abundance of select microbial populations. J. Appl. Microbiol. [Internet]. 2019; 127(1):150–158.doi: https://doi.org/p9b4

[34] Blake K, Mann M. Effect of calcium and phosphorus on the gastrointestinal absorption of ^{203}Pb in man. Environ. Res. [Internet]. 1983; 30(1):188–194. doi: https://doi.org/c92n9f

[35] Aungst BJ, Fung HL. The effects of dietary calcium on lead absorption, distribution, and elimination kinetics in rats. J. Toxicol. Environ. Health. [Internet]. 1985; 16:147–159. doi: https://doi.org/brhww5

[36] Phillips CJC., Mohamed MO, Chiy PC. Effects of duration of exposure to dietary lead on rumen metabolism and the accumulation of heavy metals in sheep. Small Rumin. Res. [Internet]. 2011; 100(2–3):113–121. doi: https://doi.org/fjp86f

[37] Deshommes E, Tardif R, Edwards M, Sauvé S, Prévost M. Experimental determination of the oral bioavailability and bioaccessibility of lead particles. Chem. Cent. J. [Internet]. 2012; 6:138. doi: https://doi.org/p9b5

[38] Pearl DS, Ammerman CB, Henry PR, Littell RC. Influence of dietary and calcium on tissue accumulation and depletion, lead metabolism and tissue mineral composition in sheep. J. Anim. Sci. [Internet]. 1983; 56(6):1416-–1426. doi: https://doi.org/p9b6

[39] Moses E, Ehireme IA. Animal exposure to lead, mechanism of toxicity and treatment strategy – A review. Clin. Case Rep. [Internet]. 2025 [cited 22 Apr 25]; 9(1):1–9. Available in: https://goo.su/NuqiW

[40] Bischoff K, Thompson B, Erb NH. Higgins WP, Ebel JG, Hillebrandt JR. Declines in blood lead concentrations in clinically affected and unaffected cattle accidentally exposed to lead. J. Vet. Diagn. Investig. [Internet]. 2012; 24(1):182–187. doi: https://doi.org/bhx9sm

[41] Valtorta SE, Litterio JN, Cerutti RD, Beldomenico HR, Coronel JE, Boggio JC. Daily rhythms in blood and milk lead toxicokinetics following intravenous administration of lead acetate to dairy cows in winter. Biol. Rhythm Res. [Internet]. 2003; 34(3):221–231. doi: https://doi.org/bgrhmc