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Kinetics of Abamectin Disposition in Blood Plasma and Milk of Lactating Dairy Sheep and Suckling Lambs

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Abamectin (ABM) has been used worldwide as an anthelmintic drug in veterinary medicine and as an agricultural pesticide. Its pharmacokinetics and permeation into milk was evaluated in dairy sheep after subcutaneous administration. ABM elimination half-lives and mean residence times were 1.7 and 3.7 days for blood plasma and 1.9 and 3.8 days for milk, respectively. The ABM milk to plasma concentration ratio (0.89) primarily depends on milk fat content. Transfer of ABM residues to suckling lambs was evaluated by determination of ABM concentration time courses in lambs' plasma. Mean maximal concentration in lambs was 1.6 μg L^{-1} at 3.3 days, and elimination half-life was 2.7 days. In ewes' plasma and milk, ABM was detected up to 23 days. Because of different pharmacokinetics, ABM exposure in lambs was almost 10% of the exposure in ewes, although the amount excreted in milk was only 1.0% of the dose.

KEYWORDS: Abamectin; avermectin; residues; pharmacokinetics; plasma; milk; sheep; lamb

INTRODUCTION

Abamectin (ABM), a natural fermentation product of the soil microorganism *Streptomyces avermitilis*, has been used worldwide since 1985 as an anthelmintic drug in veterinary medicine and as an agricultural pesticide. ABM is also used as a precursor in the production of ivermectin by catalytic hydrogenation of the C-22,23 bond (**Figure 1**). Both substances belong to the macrocyclic lactone family, with remarkably long-lasting efficacy against a broad spectrum of internal and external parasites in domestic and food producing animals. After subcutaneous administration ABM has a tremendous potency against most species of gastrointestinal nematodes (*1*), in cattle being even more effective against gastrointestinal nematodes than ivermectin (*2*). Claims against ectoparasites are more limited (*3*). In Slovenia, ABM is registered for use in cattle and sheep.

The impact of anthelmintic treatment on milk production in dairy animals, mostly cattle, has been extensively reviewed by Gross et al. (*4*). The unique broad-spectrum efficacy of macrocyclic lactone drugs against endo- and ectoparasites and long biological half-life, coupled with the low prevalence of resistance, gives them a crucial role in combating parasitic diseases in animals intended for human consumption, including small ruminants (*5*).

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In Slovenia, sheep breeding has markedly increased in the last decade. The Istrian Pramenka sheep, the breed used in the present study, is an autochthonous Slovenian milk breed, reared primarily in the pasture regions of the Karst and Istria in the Mediterranean climatic zone. It is characterized by a high milk yield of 140–150 kg year^{-1} with a high percentage of fat, protein, and dry matter content (*6*).

Partition of drugs into milk is a complex process relating to physicochemical characteristics and membrane interactions establishing a reversible equilibrium of the drug between milk and blood plasma, reflecting similar elimination profiles from these two compartments (*7*). The partitioning of hydrophobic drugs into plasma components represents a pivotal step for the drug distribution in the organism. In blood plasma, 96% of macrocyclic lactone drug is associated with lipoproteins, with a preferential binding (80–90%) to high density lipoproteins (HDL). Compared to other macrocyclic lactones ABM is more extensively (8%) distributed into very low density lipoproteins (VLDL) (*8*). Secretion of drug residues in milk is problematic for reasons of consumer safety. As for most macrocyclic lactones, ABM use in dairy animals is banned in the European Union (*9*). Associated with high lipophilicity (*10*), these compounds are extensively partitioned into milk, in lactating cattle accounting for up to 5% of the dose (*11*).

The aim of the present study was to evaluate the kinetics of ABM permeation in milk after subcutaneous administration of a therapeutic dose in dairy sheep by following the concentration time courses of ABM in blood plasma and milk. Additionally,

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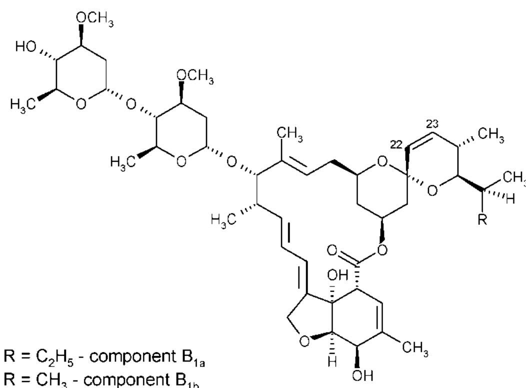

Figure 1. Structure of abamectin (ABM).

ABM exposure in suckling lambs was evaluated. Available data in the literature regarding ABM pharmacokinetics in food-producing animals are scarce. Although there are studies describing blood plasma time courses of ABM after oral (*12*) and pour-on (*13*) administration in sheep, there are almost no data on ABM disposition in milk, the composition of which is highly variable between different species. The time course of ABM in milk was studied only in lactating goats after oral administration at different dose levels and under multiple dosing regimens (*14*). To the best of our knowledge, there are also no previous data on drug exposure of suckling lambs following ABM treatment of ewes.

MATERIALS AND METHODS

Experimental Animals and Study Design. The experiment was carried out in the spring at the Centre for Sustainable Recultivation Vremščica, a department of the Veterinary Faculty of the University of Ljubljana. Eight stabled Istrian Pramenka ewes (59.5 ± 2.8 kg; 5.5 ± 2.1 years), each having a single suckling lamb (19.8 ± 1.5 kg; 6 weeks), were enrolled in the experiment. The animals were clinically healthy and parasite free, as indicated by hematological, biochemical, and fecal examinations. They had not received any avermectin drug for at least a year, nor any other drug for at least 2 months before the beginning of the experiment. During the entire experiment, the feeding regimen was as follows: all animals were fed hay and mixed fodder (maize, barley, and oily rape); lambs were nursed and additionally fed with aftermath. For all animals, water was available *ad libitum*. The animals' general health status, including measurements of body temperature, heart pulse, breathing, rumination, blood chemistry, and intestinal parasites, as well as body weight, was controlled over the entire study. Lambs were kept with their mothers, except on sampling days, when they were separated for approximately 6 h in order to collect enough milk needed for the analysis.

Six ewes were administered a single subcutaneous dose of 0.2 mg kg^{-1} of body weight of ABM from the commercially available oil preparation Abamitel L.A. containing 10 mg mL^{-1} (Krka, d.d., Novo mesto, Slovenia) in the shoulder area. Individual blood (25 mL) and milk (100 mL) samples were taken on days 0 (before administration), 1, 2, 3, 4, 5, 7, 9, 11, 14, 17, 20, 23, 26, 29, 32, 36, and 42 following ABM administration to sheep. Blood samples from lambs (10 mL) were taken on the same days, up to day 14, at the same time of the day as from their mothers. Additionally, samples from two untreated ewes kept separately and their lambs were taken for control. Blood was taken from the jugular vein in Li-heparinized vacuum tubes (Greiner, Kremsmünster, Austria). Samples were cooled to 4 °C and transported to the laboratory, where blood plasma was separated by centrifugation at 2470 ***g*** for 20 min. All samples taken were kept frozen at −20 °C until analysis. The animal experiment was approved by the Veterinary Administration of the Republic of Slovenia (323–02–187/01).

Analytical Method for ABM Determination. The concentration of ABM in an individual sheep's blood plasma and milk samples was determined using an HPLC method based on a previously used analytical procedure for determination of ivermectin in blood plasma and milk (*15–17*).

Chemicals. Pure reference standard of ABM was a gift from Krka, d.d., Novo mesto, Slovenia. Standardized solutions were prepared in acetonitrile using previously silanized glassware. All reagents were obtained from Merck (Darmstadt, Germany) and were of p.a. purity; LiChrosolv (HPLC) solvents were used for preparation of the mobile phase. Extraction columns Bakerbond with C_8 sorbent (500 mg, 6 mL) were purchased from J.T. Baker, Phillipsburg, NJ.

Extraction, Clean up, and Derivatization. Blood plasma samples (4 mL) and milk samples (5 g) were extracted with 16 and 20 mL of acetonitrile, respectively, by manual shaking for 5 min, ultrasound for 15 min (ultrasonification bath Iskra: UZ 4P and Sonis 4, Šentjernej, Slovenia), and manual shaking again for 5 min. After centrifugation at 3290 ***g*** for 10 min (centrifuge Heraeus: Minifuge 3 S-R, Osterode, Germany), 50 μL of triethylamine was added to 15 mL of acetonitrile extract, which was further diluted with distilled water to 50 mL and cleaned up using a solid phase extraction (SPE) on C_8 cartridges, previously conditioned by 5 mL of acetonitrile and 5 mL of a mixture of acetonitrile, water, and triethylamine (30:70:0.1, v/v/v). After the diluted sample extract was applied, the SPE columns were washed with 5 mL of a mixture of acetonitrile, water, and triethylamine (50:50:0.1, v/v/v) as described by Nordlander and Johnsson (*18*), followed by elution with 5 mL of acetonitrile. Eluates were concentrated until dryness at 50 °C under a nitrogen stream (evaporator Organomation: N-evap No. 111, Berlin, MA, U.S.A. and Liebisch: 2366, Bielefeld, Germany) and further derivatized at room temperature with 100 μL of *N*-methylimidazole (NMIM) solution in acetonitrile (1:1, v/v) and 150 μL of trifluoroacetic anhydride (TFAA) solution in acetonitrile (1:2, v/v) (*19*). A fluorescent aromatic derivative of ABM was formed 30 s after the NMIM and TFAA addition as described in Berendsen et al. (*20*). After dilution by 750 μL of acetonitrile, 50 μL of the final extract was injected into the HPLC system.

Chromatographic Analysis. Agilent 1100 HPLC system (Agilent, Palo Alto, CA.) was used, which consisted of a quaternary pump G1311A, vacuum degasser G1322A, automatic injector G1329A with temperature-controlled sample tray G1330A, column thermostat G1316A, fluorescence detector G1321A, and integration software ChemStation G2170AA and G2180AA. The chromatographic resolution was performed at 27 °C on a Supelcosil LC-8-DB 150 × 4.6 mm (5 μm) reversed deactivated analytical column (Supelco, Bellefonte, PA) connected to a 2 cm precolumn with the same stationary phase. The mobile phase was a mixture of acetonitrile, methanol, and water (47:47:6, v/v/v) and was pumped isocratically at a flow rate of 1.1 mL min^{-1}. Excitation and emission wavelengths were 364 and 470 nm, respectively. The main component (B_{1a}) of ABM was measured, which is also used as a marker for the presence of ABM residues in food of animal origin (*9*). The results were evaluated according to the external standard method using a standard calibration curve, constructed by plotting peak area as a function of analyte concentration.

Quality Assurance Procedures. Each sample series consisted of a negative sample to control selectivity, animal study samples, and two recovery samples. Ewes' samples were analyzed in two replicates. Recovery standard addition matched the found concentrations in biological materials under investigation. Mean measured sample concentrations were corrected for mean recovery of the respective series and used as final results.

Validation of the Analytical Method. Validation of the analytical method for ewes' matrices was performed according to the latest European Union criteria for the analysis of veterinary drugs in food, laid down by Commission Decision 2002/657/EC (*21*). The method was not validated for blood plasma of suckling lambs for ethical reasons concerning the considerable quantity of biological material required. Background in the area of ABM retention time was used to estimate selectivity. Linearity was determined by the least-squares method to calculate regression and correlation parameters between chromatographic peak areas and standard concentrations (ranges 0.00025–0.004 μg mL^{-1} and 0.001–0.15 μg mL^{-1}), and for both matrices as a correlation between measured and added concentrations (ranges 0.05–1 μg L^{-1} and 1–40 μg L^{-1} for blood plasma, and 0.05–1 μg kg^{-1} and

Table 1. Recovery, Precision, and Limit of Detection (LOD) of Abamectin (ABM) B_{1a} Determination in Ewes' Blood Plasma and Milk

parameter	blood plasma added concentration (μg L^{-1})			milk added concentration (μg kg^{-1})		
	1	5	10	1	5	10
accuracy (recovery (%) $n = 6$)	103.41	99.63	88.64	82.60	89.04	95.16
repeatability (mean found concentration CV (%) $n = 18$)	0.95	4.45	9.06	0.89	4.52	9.41
	13.8	10.7	8.2	7.8	6.5	5.6
within-laboratory reproducibility (mean found concentration, CV (%) $n = 18$)	0.94	4.72	8.77	0.93	4.46	9.41
	10.7	7.3	10.5	10.2	8.5	10.2
limit of detection (LOD)		0.02			0.04	

1–40 μg kg^{-1} for milk) of ABM. A mean recovery was evaluated with spiked blank materials at three concentration levels (1, 5, and 10 μg L^{-1} for blood plasma, and 1, 5, and 10 μg $\times$ kg^{-1} for milk), each in six replicates. The repeatability of the method was evaluated as the coefficient of variation (CV) of the determined values for measurements performed on three separate occasions close to each other (under completely equal conditions), with three concentration levels (the same as for recovery determination) in six replicates on each occasion ($n = 18$). The within-laboratory reproducibility of the method was evaluated as the CV of the determined values for measurements performed on three separate occasions (considering different analysts, chemicals, time deviation), with three concentration levels (the same as for recovery determination) in six replicates on each occasion ($n = 18$). Limit of detection (LOD) was estimated as the mean ABM concentration in the retention time window where the analyte was to be expected, which corresponded to 3 $\times$ noise.

Pharmacokinetic Analysis. ABM pharmacokinetics in ewes were assessed by fitting a one-compartment model with first order absorption and elimination to concentration-time data using WinNonlin™ Version 2.1 (Pharsight Corporation, Mountain View, CA) software. Ordinary least-squares sum was used as a criterion function in the fitting process. Goodness of fit was estimated by visual inspection of the fitted curve and correlation coefficient, which exceeded 0.95. The one-compartment model was selected on the basis of favorable Akaike Information Criterion (AIC) compared to three alternative models (one-compartment with lag time, two-compartment with and without lag time). Apparent volume of distribution divided by bioavailable fraction (V_d/F), absorption rate constant (K_a), absorption half-life (K_a-HL), elimination rate constant (K_e), elimination half-life (K_e-HL), area under the ABM concentration versus time curve extrapolated to infinity (AUC), clearance divided by bioavailable fraction (Cl/F) and mean residence time (MRT) were calculated according to conventional algorithms. Maximum concentration (c_{max}) and time to reach maximum concentration (t_{max}) were reported as observed. The same pharmacokinetic analysis was used for blood plasma and milk concentration data. Milk density of 1.036 kg L^{-1} was taken for comparison of pharmacokinetic parameters obtained from milk concentration profiles to parameters obtained from blood plasma concentration profiles (*22*).

Lambs' blood plasma concentration time data were evaluated by noncompartmental pharmacokinetic analysis. The dose of ABM received by the lamb was estimated by multiplication of an individual ewe's AUC in milk with the average milk production rate of 1 L day^{-1}.

Determination of Fat in Sheep's Milk. Analyses were performed with IR spectrometry according to IDF standard 141C:2000 (*23*) using Milcoscan FT 120 (Foss Electric, Hillerød, Denmark).

RESULTS AND DISCUSSION

Validation criteria of the procedure for determining ABM in ewes' blood plasma and milk by HPLC fluorescence met the requirements for analysis of veterinary drug residues in food of animal origin as laid down by European Union Commission Decision 2002/657/EC (*21*). Decision limit (CCα) and detection capability (CCβ) were not calculated, as they were not relevant for the purpose of this study.

To estimate the content of ABM in the blood plasma and milk samples, we quantified its major component B_{1a}, although the analytical method can also detect the minor component B_{1b}. The retention time of B_{1a} was around 5.0 min. This method is very selective, as there were no interferences at the retention time of B_{1a} in both matrices.

The method was linear for ABM B_{1a} standards (range 0.00025–0.15 μg mL^{-1}) and both matrices (range 0.05–40 μg L^{-1} for blood plasma and 0.05–40 μg kg^{-1} for milk) as proved by correlation coefficients $\geq$0.998 and $\geq$0.989, respectively. Recovery, precision and limit of detection (LOD) of ABM determination in ewes' blood plasma and milk are presented in **Table 1**. Mean recoveries over a range of 1–10 μg L^{-1} for blood plasma and 1–10 μg kg^{-1} for milk were 97.2 and 88.9%, respectively. CVs of the concentrations detected in the fortified samples were from 7.3–10.7% under within-laboratory reproducibility conditions. Because of very low noise, LOD values for blood plasma and milk were 0.02 μg L^{-1} and 0.04 μg kg^{-1}, respectively.

In accordance with Commission Decision 2002/657/EC (*21*), the quality and comparability of analytical results generated by laboratories approved for official residue control are ensured by using quality assurance systems and specifically by applying methods validated according to the guidelines laid down by this decision. Avermectins are authorized for use in food producing animals and therefore belong in group B substances of the Council Directive 96/23/EC (*24*) classification. Consequently, the presented HPLC analytical method with fluorescence detection can serve both screening and confirmation purposes for the analysis of residues in food of animal origin, including milk (*21*). This method has already been implemented and used for Slovenian statutory monitoring purposes.

On the basis of ABM concentration values in ewes' blood plasma and milk, individual concentration-time profiles following single subcutaneous administration of 0.2 mg of ABM per kg of body weight were constructed. Transmission of ABM residues to suckling lambs was evaluated by determination of blood plasma concentration profiles in individual lambs. Mean concentration time courses in the matrices investigated are presented in **Figure 2**, and the results of pharmacokinetic analysis are summarized in **Table 2**.

Following drug administration, mean maximal ABM concentrations observed (c_{max}) in ewes' blood plasma and milk were 30.9 and 26.8 μg L^{-1}, respectively, at day 1.7 and 2.0 (t_{max}), respectively. The highest ABM concentrations detected in blood plasma and milk samples were 42.0 and 37.5 μg L^{-1}, respectively, found in the same animal on the first day post-treatment. Mean ABM c_{max} in blood plasma was approximately 3-fold higher compared to ivermectin (*25*) and 2-fold higher compared to doramectin (*26*) as observed in our previous studies performed under the same conditions, including animal species and

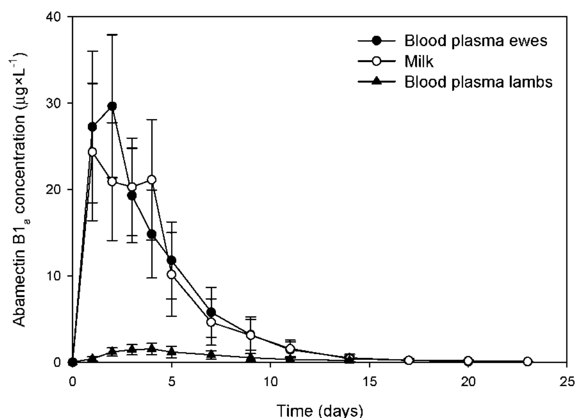

Figure 2. Abamectin (ABM) concentration profiles (mean ± S.D.) in blood plasma and milk of sheep ($n = 6$) following subcutaneous administration of 0.2 mg ABM per kg of body weight, and in blood plasma of their suckling lambs.

Table 2. Abamectin (ABM) B_{1a} Pharmacokinetics (mean ± S.D.) in 6 Sheep Based on Blood Plasma and Milk Concentration Data Following a Single Subcutaneous Administration of 0.2 mg of ABM per kg of Body Weight and in Blood Plasma of Six Suckling Lambs[a]

pharmacokinetic parameter	ewes[b]		lambs[c]
	blood plasma	milk	blood plasma
t_{max} (day)[d]	1.7 ± 0.5	2.0 ± 1.5	3.3 ± 0.8
c_{max} (μg L^{-1})[d]	30.9 ± 8.7	26.8 ± 7.0	1.6 ± 0.6
V_d/F (L kg^{-1})	3.7 ± 1.3	n.a.	2.0 ± 0.7
K_a (day^{-1})	0.94 ± 0.52	1.12 ± 0.58	n.a.
K_a-HL (day)	0.90 ± 0.41	0.75 ± 0.31	n.a.
K_{el} (day^{-1})	0.44 ± 0.10	0.39 ± 0.09	0.31 ± 0.13
K_{el}-HL (day)	1.65 ± 0.44	1.85 ± 0.43	2.73 ± 1.48
AUC (μg day L^{-1})	131.2 ± 31.9	115.6 ± 31.9	11.1 ± 7.8
Cl/F (L day^{-1} kg^{-1})	1.6 ± 0.6	n.a.	0.6 ± 0.5
MRT (day)	3.68 ± 0.84	3.75 ± 0.88	6.02 ± 1.95
M/P		0.89 ± 0.13	
dose fraction (%)[e]		1.0 ± 0.3	

[a] t_{max}, time to maximum concentration; c_{max}, maximum concentration; V_d/F, apparent volume of distribution divided by bioavailable fraction; K_a, absorption rate constant; K_a-HL, absorption half-life; K_{el}, elimination rate constant; K_{el}-HL, elimination half-life; AUC, area under the ABM concentration versus time curve; Cl/F, clearance divided by bioavailable fraction; MRT, mean residence time; M/P, milk to plasma concentration ratio; n.a., not applicable. [b] Pharmacokinetic parameters were assessed by fitting a one-compartment model. [c] Pharmacokinetic parameters were assessed by noncompartmental analysis. [d] Reported as observed. [e] Dose fraction recovered in milk.

breed, physiological conditions, nutrition, mode of drug administration, sampling schedule, residue, and pharmacokinetic analysis. Henceforth, t_{max} ABM concentrations gradually declined with time and fell below LOD for both ewes' matrices from day 17 onward. On day 26, ABM was found in milk in only one of six ewes but was below LOD in the next samples, taken after 29 days.

In ewes, mean excretion time for ABM concentration to fall below LOD was 22.5 and 23 days for blood plasma and milk, respectively. The presented results confirm the long withholding time of ABM in the organism, which was similar to ivermectin (*25*), but was considerably shorter than doramectin (*26*). The difference in withholding time is related to lipophilicity, as the octanol/water partition coefficient of doramectin is the highest of the three avermectins studied. Pharmacokinetic parameters of ABM in sheep substantially differed from other species previously studied.

ABM exposure in sheep can be evaluated by comparison of AUC to previously reported values for different animal species.

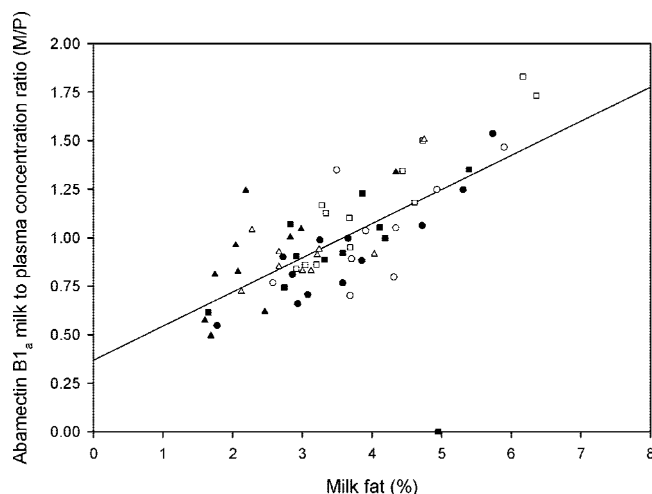

Figure 3. Relationship between abamectin milk to plasma concentration ratio (M/P) and milk fat content in six sheep following a single subcutaneous administration of 0.2 mg per kg of body weight. Each sheep is marked with a different symbol.

In our study in sheep, AUC was approximately 2-fold lower than in pigs after intramuscular administration of 0.3 mg kg^{-1} (*27*) and in calves after subcutaneous administration of 0.2 mg kg^{-1} (*28*). However, ABM exposure in sheep found in our study following subcutaneous administration of 0.2 mg kg^{-1} was similar to exposure in a previously reported study (*13*) with pour-on administration of 0.5 mg kg^{-1}, in which approximately 3-fold higher AUC was achieved with 2.5-fold higher dose. In our study in sheep, ABM blood plasma concentrations were above the minimal effective concentration of 1 μg L^{-1} (*29*), between 7 and 14 days (mean 10.5 days), which is again 40% shorter than in calves with the same dosing (*28*).

As previously observed for ivermectin (*25*) and doramectin (*26*), ABM milk to plasma concentration ratio M/P depends on milk fat content as demonstrated in **Figure 3**. However, comparison of the three avermectins indicates that the extent of ABM permeation into milk ($M/P = 0.89 \pm 0.13$) is lower than permeation of ivermectin ($M/P = 1.83 \pm 0.54$; $p < 0.005$ independent samples t test) and doramectin ($M/P = 1.40 \pm 0.51$; $p < 0.05$ independent samples t test). We assume that the reason for this difference originates not only in lipophilicity but also in a specific feature of their chemical structure. Looking to increase macrocyclic lactone use in dairy animals, Shoop et al. (*30*) reported that saturation of the C-22,23 bond of the macrocyclic lactone molecule facilitated partition into milk with concentrations in milk higher than in plasma, leading to M/P greater than 1. Compounds that were not saturated at the C-22,23 position, such as the 4″-epi-acetylamino or 5-oxime derivative of ivermectin, have M/P ratios less than 1. This finding was a milestone in the development of eprinomectin, which is used in lactating dairy cattle with zero milk withholding time. Taking this into account, we can explain the difference in M/P between ivermectin (single C-22,23 bond) and ABM (double C-22,23 bond). Additionally, lipophilicity appears to be important, as M/P was also high for more lipophilic doramectin with double C-22,23 bond.

By multiplying the AUC value for milk data with the average milk yield per sheep of 1 L day^{-1}, it can be estimated that 1.0 ± 0.3% (mean ± S.D.) of the dose administered to ewes was excreted in milk and transferred to suckling lambs, which was lower compared to 5% for moxidectin in a study in a suckling calf by Alvinerie et al. (*31*). In the latter study, however, the fraction of the dose received by suckling was estimated

indirectly from the calf/cow ratio of areas under the plasma concentration curves. In lambs' blood, a plasma mean c_{max} of 1.6 μg L^{-1} was observed at 3.3 days (t_{max}) following drug administration to ewes. Maximal individual concentration detected was 2.5 μg L^{-1}. In spite of the fact that only 1.0% of the ABM dose was excreted in milk, the mean ratio of AUC for blood plasma between ewes and lambs was almost 10%. Taking into consideration the fact that the bioavailability after oral administration is lower compared to subcutaneous administration, the difference in plasma AUC between lambs and ewes cannot be explained by the allometric scaling approach alone. Evidently, physiological differences between adult animals and lambs as discussed by Bogan and McKellar (*32*) have an important role. On day 14, residual ABM concentrations were still detected in five out of six lambs. This finding has a valuable implication for consumer safety, as the majority of lambs are slaughtered within 30 days of weaning in Slovenia.

A toxicological ADI of 0.00025 mg per kg of body weight, i.e., 15 μg per person for a 60 kg adult, was previously established for ABM by the EMEA Committee for Veterinary Medicinal Products (CVMP) by applying a safety factor of 200 to the NOEL of 0.05 mg per kg of body weight per day in a study in CF-1 mice, based on maternotoxicity (*33, 34*). Following additional data directly relevant for human risk assessment, acquired from a 1-year repeated-dose study in dogs, EMEA CVMP established a revised ADI of 2.5 μg per kg of body weight (150 μg per person), by applying a safety factor of 100 to the NOEL of 0.25 mg per kg of body weight per day. On the basis of existing maximum residue levels (MRLs), total maximum theoretical intake from both veterinary and pesticidal (animal consumption of fodder containing ABM) use should not exceed around 13% of the revised ADI (*35, 36*). There are no MRLs for ABM in milk (bovine or ovine) in the EU regarding Regulation (EEC) No. 2377/90 (*9*). This means that ABM-based products may not be used in animals that are producing milk for human consumption and that residues in milk are not allowed (zero tolerance). However, in cases of emergency (other products not available, life-threatening situation for the animal, etc.; this is not necessarily the same in all EU Member States), it could mean that such a product can be conditionally administered to milk-producing animals, provided that the milk is kept out of the normal distribution channel for human consumption. In such off-label use, also including misuse and abuse of the product, the withdrawal period must be judged by a veterinarian (*37*).

The presented results suggest that for reasons of consumer safety, milk from ABM-treated animals should be excluded from the food chain, in particular from production of fat products like cheese, for about 1 month following treatment with ABM.

ABBREVIATIONS USED

ABM, abamectin; HDL, high density lipoproteins; VLDL, very low density lipoproteins; MRL, maximum residue level; HPLC, high performance liquid chromatography; CV, coefficient of variation; M/P, milk to plasma concentration ratio; ADI, acceptable daily intake value; NOEL, no observed effect level; EMEA, European Agency for the Evaluation of Medicinal Products; CVMP, Committee for Veterinary Medicinal Products.

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