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RESIDUE EVALUATION OF CERTAIN VETERINARY DRUGS

Joint FAO/WHO Expert Committee on Food Additives

75th meeting 2011

World Health Organization

Food and Agriculture Organization of the United Nations

FAO JECFA Monographs 12

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Joint FAO/WHO Expert Committee on Food Additives

75th Meeting

Rome, Italy, 8–17 November 2011

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
WORD HEALTH ORGANIZATION
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LIST OF PARTICIPANTS

Seventy-fifth Meeting of the
Joint FAO/WHO Expert Committee on Food Additives (JECFA)
Rome, Italy, 8–17 November 2011

Members

Professor A. Anadón, Department of Toxicology and Pharmacology, Faculty of Veterinary Medicine, Universidad Complutense de Madrid, Madrid, Spain

Dr D. Arnold, Consultant, Berlin, Germany (Vice-Chair)

Professor A.R. Boobis, Centre for Pharmacology and Therapeutics, Department of Experimental Medicine, Division of Medicine, Faculty of Medicine, Imperial College London, London, England, UK (Chair)

Dr R. Ellis, Consultant, Myrtle Beach, SC, USA (Joint Rapporteur)

Dr A. Fernández Suárez, Ciencias Veterinarias, Universidad del Salvador, Buenos Aires, Argentina

Dr L.G. Friedlander, Office of New Animal Drug Evaluation, Center for Veterinary Medicine, Food and Drug Administration, Department of Health and Human Services, Rockville, MD, USA

Dr K.J. Greenlees, Office of New Animal Drug Evaluation, Center for Veterinary Medicine, Food and Drug Administration, Department of Health and Human Services, Rockville, MD, USA (Joint Rapporteur)

Professor J. Palermo-Neto, Department of Pathology, Faculty of Veterinary Medicine, University of São Paulo, São Paulo, Brazil

Professor Emeritus L. Ritter, University of Guelph, Guelph, Ontario, Canada

Dr P. Sanders, Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES) [National Reference Laboratory for Veterinary Drug Residues and Antimicrobial Resistance], Fougères, France

Professor G.E. Swan, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa[1]

Secretariat

Dr J. Boison, Centre for Veterinary Drug Residues, Canadian Food Inspection Agency, Saskatoon, Saskatchewan, Canada (FAO Expert)

Dr A. Bruno, Joint FAO/WHO Food Standards Programme, Nutrition and Consumer Protection Division, Food and Agriculture Organization of the United Nations, Rome, Italy (FAO Codex Secretariat)

Dr C.E. Cerniglia, Division of Microbiology, National Center for Toxicological Research, Food and Drug Administration, Department of Health and Human Services, Jefferson, AR, USA (WHO Temporary Adviser)

Dr P.L. Chamberlain, Office of the Chief/Office of the Chief Scientist/Office of Counterterrorism and Emerging Threats, Food and Drug Administration, Department of Health and Human Services, Silver Spring, MD, USA (WHO Temporary Adviser)

Dr S. Ghimire, Veterinary Drugs Directorate, Health Canada, Ottawa, Ontario, Canada (WHO Temporary Adviser)

Dr N. Jarrett, European Medicines Agency, London, England, UK (WHO Temporary Adviser)

Professor S.H. Jeong, Department of Applied Biotoxicology, Hoseo University, Hoseo Toxicology Research Centre, Asan City, Chungnam, Republic of Korea (WHO Temporary Adviser)

Professor B. Le Bizec, Laboratoire d'Étude des Résidus et des contaminants dans les aliments (LABERCA), École Nationale Vétérinaire, Agroalimentaire et de l'Alimentation Nantes Atlantique (ONIRIS), Nantes, France (FAO Expert)

Dr K. Ogawa, Division of Pathology, Biological Safety Research Center, National Institute of Health Sciences, Tokyo, Japan (WHO Temporary Adviser)

Dr F. Ramos, Bromatology, Pharmacognosy and Analytical Sciences Group, Pharmacy Faculty, Coimbra University, Coimbra, Portugal (FAO Expert)

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1. Dr Swan was invited but unable to attend the meeting.

Dr G. Roberts, Consultant, Preston, Victoria, Australia (WHO Temporary Adviser)

Ms M. Sheffer, Orleans, Ontario, Canada (FAO/WHO Editor)

Dr A. Tritscher, Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland (WHO Joint Secretary)

Dr P. Verger, Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland (WHO Joint Secretary to JMPR)

Dr A. Wennberg, Nutrition and Consumer Protection Division, Food and Agriculture Organization of the United Nations, Rome, Italy (FAO Joint Secretary)

ABBREVIATIONS

ADI	Acceptable daily intake
ADME	Absorption, distribution, metabolism and excretion
AOAC	AOAC International
AUC	Area under the curve
BLQ	Below limit of quantitation
bw	body weight
CAC	Codex Alimentarius Commission
CAS	Chemical Abstracts Service
CCRVDF	Codex Committee on Residues of Veterinary Drugs in Foods
CL	Clearance rate
C_{max}	Maximum concentration
CR	Clearance (Renal)
CV	Coefficient of variation
C_{vr}	Reproducabilty
CRC	Controlled Release Capsule
ECD	Electron capture detector
EDI	Estimated daily intake
F	Bioavailability
FAO	Food and Agriculture Organization of the United Nations
GC	Gas chromatography
GLP	Good laboratory practice
h	hour
HPLC	High pressure liquid chromatography
i.m.	intramuscular [injection]
IR	Infrared
IUPAC	International Union of Pure and Applied Chemistry
i.v.	intravenous
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
kg	kilogram (10^3 g)
L	litre
LC	Liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantitation
LSC	liquid scintillation counting
µg	microgram (10^{-6} g)
mg	milligram (10^{-3} g)
min	minimum or minute
ml	millilitre
MRL	maximum residue limit

MRT	mean residence time
MS	mass spectrometry
MW	molecular weight
ng	nanogram (10^{-9} g)
NOAEL	No observed adverse effect level
NQ	Non-quantifiable
QA	Quality assurance
QC	Quality control
RP	Reverse phase
rsd	Repeatability standard deviation
R_t	retention time
s.c.	subcutaneous [injection]
SD	Standard deviation
SPE	Solid phase extraction
$t_{½}$	Half life
TR	Total residue
TMDI	Theoretical maximum daily intake
TRR	total radiolabelled residues
UV	ultraviolet
VD	volume of distribution
VD_{SS}	volume of distribution at steady-state
WHO	World Health Organization

INTRODUCTION

The monographs in this volume of the FAO JECFA Monographs on the residues of, statements on, or other parameters of the veterinary drugs on the agenda were prepared by the invited experts for the Seventy-fifth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), held in Rome, Italy, 7–17-November 2011. This was the nineteenth meeting of JECFA convened specifically to consider residues of veterinary drugs in food-producing animal species. The Committee had evaluated residues of veterinary drugs at its 12th, 26th, 27th, 32nd, 34th, 36th, 38th, 40th,42nd, 43rd, 45th, 47th, 48th, 50th, 52nd, 54th, 58th, 60th, 62nd, 66th and 70th meetings (JECFA, various dates 1969–2010). The tasks for the Committee were to further elaborate principles for evaluating the safety of residues of veterinary drugs in food and for establishing acceptable daily intakes (ADIs) and recommend maximum residue limits (MRLs) for substances on the agenda when they are administered to food-producing animals in accordance with good veterinary practice in the use of veterinary drugs. The enclosed monographs provided the scientific basis for the recommendations of MRLs.

There is an important feature to bring to the attention of readers. This volume of the FAO JECFA Monographs is the third in a new format for the presentation of monographs from meetings of the Committee specifically devoted to residues of specific veterinary drugs in food. It was also the seventh meeting of JECFA subsequent to the completion of the workshop to update the principles and methods of risk assessment for MRLs for pesticides and veterinary drugs, held jointly by FAO/RIVM/WHO, in Bilthoven, The Netherlands, 7–11 November 2005. The outcomes of this workshop are incorporated in the Environmental Health Criteria, No. 240, publication *Principles and methods for the risk assessment of chemicals in food*, WHO, 2009. Specifically, the Committee continued to implement some of the more significant recommendations in the workshop report, including the concept of using median residue values to estimate daily intakes of residues of veterinary drugs in food for chronic exposure intake estimates.

Background

In response to the growing use of veterinary medicines in food animal production systems internationally and the potential implications for human health and fair trading practices, a Joint FAO/WHO Expert Consultation on Residues of Veterinary Drugs was convened in Rome in November 1984 (FAO/WHO, 1985). One of the major recommendations of this consultation was the establishment of the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) and the periodic convening of an appropriate expert body to provide independent scientific advice to this Committee and to member countries of FAO and WHO. At its first session, in Washington, DC, in November 1986, the CCRVDF reaffirmed the need for such a scientific body and made a number of recommendations and suggestions to be considered by JECFA (CCRVDF, 1986). In response to these recommendations, the 32nd JECFA meeting was devoted entirely to the evaluation of residues of veterinary drugs in food—a new responsibility for the Joint FAO/WHO Expert Committee on Food Additives. Nineteen such meetings of JECFA have been held prior to the meeting of JECFA reported here.

75th Meeting of JECFA

The present volume, in the new format, contains monographs on the residue data of seven of the substances scheduled for evaluation at the 75th Meeting of the Committee. Of the substances on the agenda, four were new evaluations (amoxicillin, apramycin, derquantel and monepental) and three were re-evaluations (monensin, narasin and triclabendazole). The re-evaluation of narasin was for a suitable analytical method in cattle tissues only. One substance, ivermectin, was originally scheduled for review by the Committee; however, there was no submission of new information regarding residues in food-producing animals on which to base any reconsideration of MRLs. The Committee noted that before it would re-evaluate the residue depletion of ivermectin and propose updated MRLs, it would need a submission indicating that a suitably validated analytical method with a limit of quantitation (LOQ) in the low µg/kg range for the marker residue has been used in the conduct of

depletion studies in fat, kidney, liver and muscle tissues of animals for which MRLs are requested. A literature review on the relevant toxicology to reconsider the acceptable daily intake (ADI) of ivermectin was conducted and a toxicological summary report was prepared for the Committee.

The monographs are prepared in a uniform format consistent with the data provided and the specific request for risk assessment by CCRVDF. The format includes identity of substance, residues in food and their evaluation, metabolism studies, tissue residue depletion studies, methods of residue analysis, a final appraisal of the study results, and if appropriate, recommendations on MRLs. A summary of the recommendations on compounds on the agenda and further information required is included in Annex 1. In addition, a summary of JECFA evaluations of residues of veterinary drugs in foods from the 32nd meeting to the present 75th meeting can be found in Annex 2.

The monographs and general considerations on risk assessment principles of this volume must be considered in the context of the full report of the meeting, which will be published in the *WHO Technical Report Series*.

On-line editions of *Residues of some veterinary drugs in animals and foods* (from FAO JECFA Monographs and *FAO Food and Nutrition Paper*, No. 41) are available. The monographs and statements that have been published in FAO JECFA Monographs No. 2 and this volume, as well as those published in *FAO Food and Nutrition Paper*, No. 41 (sixteen volumes since 1988) are all available online at http://www.fao.org/ag/agn/jecfa-vetdrugs/search.html. The search interface is available in five languages (Arabic, Chinese, English, French and Spanish) and allows searching for compounds, functional classes, ADI and MRL status.

Contact and feedback

More information on the work of the Committee is available from FAO.

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Amoxicillin

First draft prepared by
Fernando Ramos, Coimbra, Portugal
Joe Boison, Saskatoon, Canada
and
Lynn G. Friedlander, Rockville, MD, USA

IDENTITY

International Non-proprietary names (INN): Amoxicillin, formerly Amoxycillin

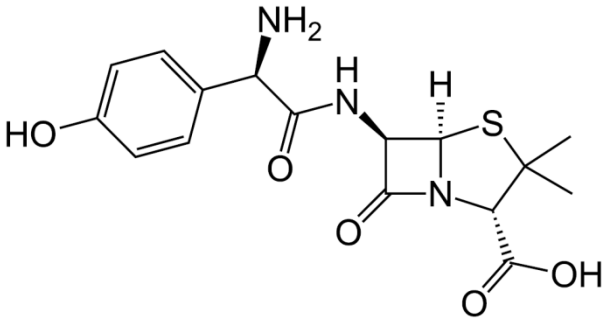
Synonyms: Amox; AMC; Amoxicillin trihydrate; Amoxicillin anhydrous; Amoxycillin trihydrate; D-Amoxicillin; p-Hydroxyampicillin

IUPAC Names: (2S,5R,6R)- 6-{[(2R)-2-amino- 2-(4-hydroxyphenyl)- acetyl]amino}- 3,3-dimethyl- 7-oxo- 4-thia- 1-azabicyclo[3.2.0]heptane- 2-carboxylic acid

[2S - [2α,5α,6β(S*)]] - 6 - [[Amino (4 - hydroxyphenyl)acetyl]amino] - 3,3 - dimethyl - 7 - oxo - 4 - thia - 1 – azabicyclo [3.2.0] heptane - 2 - carboxylic acid

Chemical Abstract Service No.: Amoxicillin: 26787-78-0, Amoxicillin trihydrate: 61336-70-7

Structural formula of main components:



Molecular formula: $C_{16}H_{19}N_3O_5S$

Molecular weight: Amoxicillin: 365.40; Amoxicillin trihydrate: 419.41

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Amoxicillin

Appearance: Powder/Crystalline solid

Melting point: 194°C

pH: 4.4–4.9 (0.25% w/v solution)

Optical rotation: +290°–315°

Solubility: 3430 mg/L water

UV_{max}: 272 nm (water)

Partition coefficient: -2.69

Stability to acids and bases: Amoxicillin is stable in the presence of gastric acid

RESIDUES IN FOOD AND THEIR EVALUATION

Conditions of use

Amoxicillin is a broad-spectrum, pharmacologically active beta-lactam antibiotic effective against Gram-positive and Gram-negative bacteria. Amoxicillin is stable in the gastro-intestinal tract and has higher absorption than naturally occurring penicillins when administrated orally. Amoxicillin is a widely used antibiotic in human and veterinary medicine for the treatment and prevention of respiratory, gastrointestinal, urinary and skin bacterial infections due to its pharmacological and pharmacokinetic properties (Sousa, 2005). Amoxicillin is de-activated by bacterial β-lactamase or penicillinases. In human medicine amoxicillin is commonly used in combination with clavulanic acid, a penicillinase inhibitor; it is not normally used with clavulanic acid in veterinary use.

Amoxicillin is used in many domestic and food animals, including cats, dogs, pigeons, horses, broiler chickens, pigs, goats, sheep, pre-ruminating calves (including veal calves) and cattle. In dogs and cats, amoxicillin is used in respiratory and urinary infections and in soft tissue wounds caused by Gram-positive and Gram-negative pathogenic bacteria (Pfizer, 2004). In poultry, amoxicillin is used for the treatment of susceptible infections of the alimentary, urogenital and respiratory tracts (APVMA, 2007). In pigs, amoxicillin is used to treat major respiratory tract pathogens, mainly caused by *Actinobacillus pleuropneumoniae*, *Streptococcus suis* and *Pasteurella multocida*. Amoxicillin also is used against some digestive and urinary tract pathogens, such as *Escherichia coli* and *Streptococcus suis* (Hernandez *et al.*, 2005; Reyns *et al.*, 2008a). In sheep, amoxicillin is used for the treatment of bacterial pneumonia due to *Pasteurella* spp. and *Haemophilus* spp. (FDA, 1999). In goats, amoxicillin is indicated for the treatment of respiratory tract infections caused by, among other microorganisms, *Mannheimia haemolytica, P. multocida, H. somnus,* but not for penicillinase-producing *S. aureus* (Baggot, undated). Amoxicillin also is used in pre-ruminating calves for treatment of bacterial enteritis due to *E. coli*, and in cattle for treatment of respiratory tract infections, including shipping fever and pneumonia due to *P. multocida*, *M. haemolytica, Haemophilus* spp., *Streptococcus* spp. and *Staphylococcus* spp., and for acute necrotic pododermititis (foot rot) due to *Fusobacterium necrophorum* (FDA, 2011). Amoxicillin is also approved for use in lactating dairy cows by intramammary infusion with a suspension of amoxicillin trihydrate containing the equivalent of 62.5 mg of amoxicillin per disposable syringe for each infected quarter (Schering-Plough, 2007).

Dosage

In food-producing animals, amoxicillin is approved for use as amoxicillin trihydrate for oral suspensions equivalent to 40 mg amoxicillin twice daily for piglets under 4.5 kg; a soluble powder of amoxicillin trihydrate at 400 mg/45.5 kg body weight (bw) twice daily for pre-ruminating calves, including veal calves, administered by drench or by mixing in milk; amoxicillin trihydrate boluses containing 400 mg of amoxicillin per 45.5 kg bw for pre-ruminating calves, including veal calves; and as a sterile amoxicillin trihydrate powder for use as a suspension at 6.6–11 mg/kg bw once a day, administered by intramuscular (i.m.) or subcutaneous (s.c.) injection in cattle. For sheep, amoxicillin is approved for use as a sterile i.m. injection suspension containing 50 mg/ml at a dose rate of 7 mg/kg bw once a day; as a 150 mg/ml long-acting amoxicillin trihydrate oily i.m. injection suspension at 15 mg/kg bw every two days; and as a 200 mg/ml i.m. injection at 1 ml/20 kg bw for cattle, sheep and pigs (Virbac, 2008, 2011).

PHARMACOKINETICS AND METABOLISM

Pharmacokinetics in laboratory animals

Rats

Amoxicillin was administered to 11 rats at 50 mg/kg bw as a bolus dose. Microdialysis samples were collected over 180 minutes to determine the amount of unbound drug in blood and muscle (Marchand *et al.*, 2005). A two-compartment pharmacokinetic model adequately described the unbound amoxicillin concentration-time profiles in both matrices. The results obtained are represented in

Figure 1.1. Amoxicillin was distributed rapidly and extensively within muscle and interstitial fluid, indicating that alterations in muscle blood flow seem unlikely to have a major effect on drug distribution characteristics.

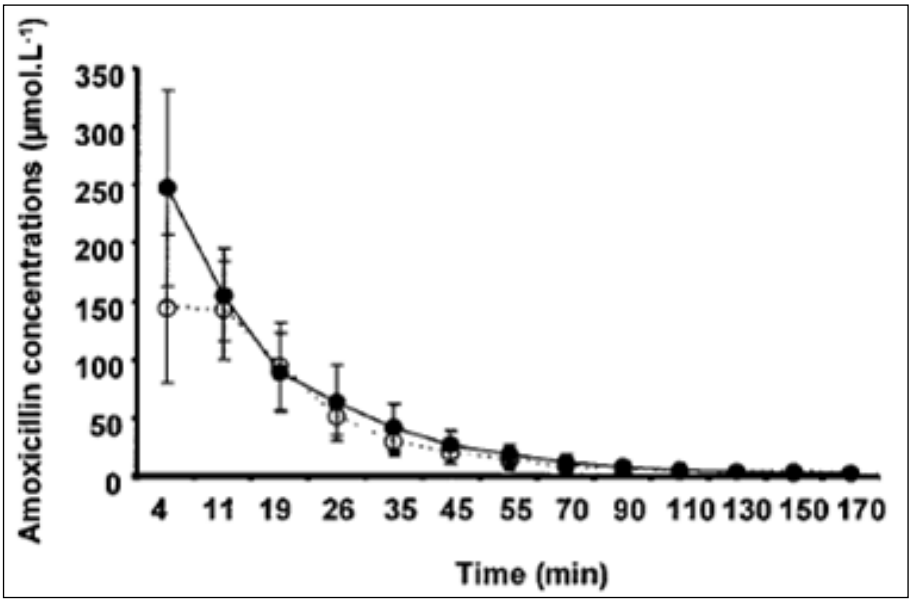


Figure 1.1. Unbound amoxicillin concentrations in blood and muscle of rats after intravenous (i.v.) bolus administration of amoxicillin at 50 mg/kg bw.
NOTES: Concentrations (mean ± SD) in blood (solid circles and solid line, n=11) and in muscle (open circles and dashed line, n=11)

Two pharmacokinetic studies were conducted to investigate the distribution of amoxicillin in rat tissues. In a Good Laboratory Practice (GLP)-compliant study using 12 healthy male Wistar rats, 3 h after a single oral administration of amoxicillin (15 or 60 mg/kg) the drug was distributed extensively in the microvilli, nuclei and cytoplasm of the absorptive epithelial cells of the intestine, in the cytoplasm and nuclei of the hepatocytes and on the luminal surface of the capillaries, intercalated portions, and interlobular bile ducts. Although almost no amoxicillin could be detected 6 h post-administration in either the intestine or the liver, it persisted until 12 h in the kidney (Fujiwara *et al.*, 2011). The second study (non-GLP-compliant) reported that, after a single oral dose of amoxicillin at 100 mg/kg to 6 rats, the drug distributed preferentially to liver and kidney (Sakamoto, Hirose and Mine, 1985).

Dogs

Six dogs were dosed orally with three formulations of amoxicillin to evaluate the effect of drug formulation on oral bio-availability: a 60 ml suspension administered by an intragastric tube; 3 ml of amoxicillin drops; or in tablet form. The liquid forms of the drug tended to be more readily absorbed than the tablets (i.e. higher bio-availability) in comparison with that calculated for the suspension (76.8 ± 16.7%) and the drops (68.2 ± 25.8%) versus the tablets (64.2 ± 17%). However, the differences between their pharmacokinetic parameters (C_{max}, t_{max} and AUC) were not statistically significant. The drops and tablets had similar pharmacokinetic profiles in the dogs and are regarded as equivalent in this species (Kung and Wanner, 1994).

Among a variety of species tested, amoxicillin distribution was independent of the binding percentage to plasma proteins (<40% in human, dog, rabbit, rat and mouse) (Sakamoto, Hirose and Mine, 1985).

Pharmacokinetics in food-producing animals

Fish

A study was conducted to determine amoxicillin residues in catfish muscle after oral administration (Ang *et al.*, 2000). Fish weighing 0.5–1.0 kg were maintained in indoor tanks prior to treatment. Using

a plastic pipette, 110 mg of amoxicillin/kg bw was administrated. Five fish were collected at each time interval for depletion periods up to 72 h post-dosing. Table 1.1 indicates the amoxicillin contents of individual fish after oral administration of the drug and depletion. All samples were analysed by a HPLC-Fluorescence method with a limit of quantitation limit (LOQ) of 1.2 µg/kg. Amoxicillin residues depleted rapidly from catfish during the first 24 h. After that the concentrations were <10 µg/kg, decreasing to <1.2 µg/kg after 72 h.

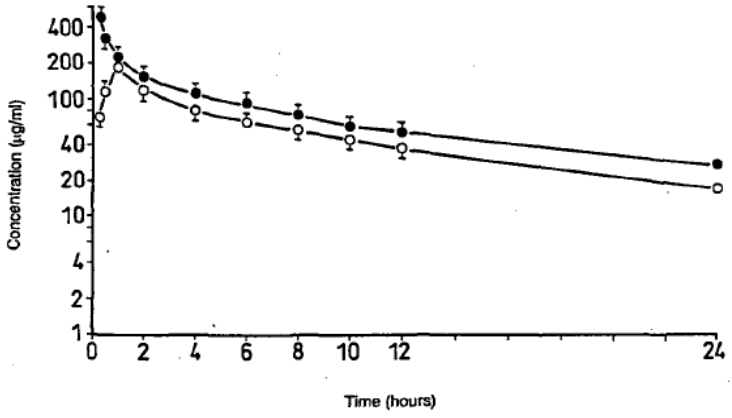
Table 1.1. Amoxicillin concentration in individual fish after oral administration of 110 mg/kg bw

Depletion time (h)	Fish weight (kg)	Mean concentration of amoxicillin (µg/kg)
6	0.76	64.2
	0.56	50.6
	0.38	60.5
	0.48	40.0
	0.66	297
24	0.38	<LOQ
	0.36	7.3
	0.32	3.7
	0.44	7.0
	0.52	7.9
48	0.50	<LOQ
	0.46	1.4
	0.54	6.9
	0.70	2.8
	0.38	1.9
72	0.48	<LOQ
	0.30	<LOQ
	0.44	<LOQ
	0.36	<LOQ
	0.36	<LOQ

Chicken

Amoxicillin was given to two groups of eight chickens at a dose of 10 mg/kg bw, intravenously or orally (Anadón *et al.*, 1996). Blood samples were collected at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h after drug administration. Plasma was separated and analysed by HPLC with UV detection. As can be seen in Figure 1.2, elimination profiles of amoxicillin were similar when administrated either i.v. or oral.

Figure 1.2. Plasma concentration of amoxicillin in chickens after intravenous (●) or oral (o) administration of 10mg/kg bw



Following oral administration, the maximum plasma concentration occurred at 1.00 ± 0.06 h with a C_{max} of 160.40 ± 4.67 µg/ml (Table 1.2). Amoxicillin concentrations in plasma declined slowly and concentrations greater than 15 µg/ml persisted up to 24 h after oral administration (Figure 1.2). The values of the kinetic parameters that describe the absorption and disposition kinetics of amoxicillin are given in Table 1.2.

Table 1.2. Pharmacokinetic parameters (mean ± SD) of amoxicillin in eight chickens after intravenous or oral dosing of 10 mg/kg bw

Parameter	Intravenous	Oral
A_1 (µg/ml)	850.23 ± 21.95	220.04 ± 43.30
A_2 (µg/ml)	182.12 ± 8.72	107.53 ± 7.56
A_3 (µg/ml)		342.54 ± 44.79
α (h^{-1})	3.05 ± 0.11	0.77 ± 0.11
β (h^{-1})	0.086 ± 0.003	0.078 ± 0.005
K_a (h^{-1})		2.39 ± 0.13
$t_{½}\alpha$ (h)	0.23 ± 0.01*	1.00 ± 0.10
$t_{½}\beta$ (h)	8.17 ± 0.31	9.16 ± 0.60
$t_{½a}$ (h)		0.30 ± 0.02
$V_{d(area)}$ (L/kg)	0.049 ± 0.002	0.054 ± 0.003
$V_{d(ss)}$ (L/kg)	0.042 ± 0.002	
K_{12} (h^{-1})	2.09 ± 0.09	0.31 ± 0.07
K_{21} (h^{-1})	0.61 ± 0.03	0.37 ± 0.04
K_{10} (h^{-1})	0.43 ± 0.03	0.16 ± 0.01
AUC (mg/h/L)	2449.3 ± 174.8	1534.6 ± 114.9
F (%)		63.00 ± 4.58
MRT (h)	10.46 ± 0.51	12.26 ± 0.81
CL (L/h/kg)	0.004 ± 0.001	0.004 ± 0.001
K_{12}/K_{21}	3.45 ± 0.12	0.83 ± 0.12
K_{12}/K_{10}	5.02 ± 0.50	1.91 ± 0.30
K_{21}/K_{10}	1.48 ± 0.17	2.40 ± 0.28
C_{max} (µg/ml)		160.40 ± 4.67
T_{max} (h)		1.00 ± 0.06

NOTES: * = Significantly different between dosing routes (P<0.05)

Cattle

Six calves were fed milk replacer containing 0.25, 1.0 or 2.0 µg of amoxicillin/ml at 6% body weight twice daily, for three consecutive feedings (Musser *et al.*, 2001). Amoxicillin was quantified in serum and urine 3, 6, 9 and 15 h after drinking medicated milk replacer. By 24 h after the final feeding, no amoxicillin was detected in urine.

In a study with 8 pre-ruminating calves, three amoxicillin sodium preparations were compared for urinary excretion related to serum concentrations following i.m. administration (Palmer, 1975a). Although the serum profiles were different, renal clearance of approximately 200 ml/minute was observed at 2–8 h post-treatment and 48–52% of the administrated dose was recovered in the urine collected from 0–8 h post-treatment.

In the first formulation (aqueous suspension), 3 pre-ruminating calves received a dose of 7 mg/kg bw. An additional 3 pre-ruminating calves were treated with a 10.5 mg/kg bw oily suspension and the other 2 pre-ruminating calves were treated with a 7 mg/kg bw aqueous solution. Urine samples were collected at 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h. Total urine was collected for time periods 1–2 h, 4–6 h, 6–8 h and 8–24 h. Blood concentrations from the aqueous suspension produced mean peak serum concentrations of 2.0–2.5 µg/ml that was sustained for 6 h, declining to 1.5 µg/ml at 8 h. Animals

treated with the oily suspension showed a similar profile, with peak mean serum levels of 3.0 µg/ml at 2–3 h post dosing.

Pre-ruminating calves treated with the aqueous solution showed a peak mean serum concentration of 7.0–7.5 µg/ml 15 minutes post-treatment, and rapidly declined below the other formulations at 3 h post-treatment. Urine collections showed that 50–60% of the drug could be recovered from the urine in the 24 h following i.m. administration independent of the formulation used, with the majority of the excreted dose recovered in the first 8 h (48–52%). The quantity of amoxicillin excreted was proportional to the serum amount for a given urine collection period. Rates of renal plasma clearance were calculated (approximately 200 ml/min in plasma) for each product tested.

In a study of 16 pre-ruminating calves, amoxicillin was administered orally at 7 mg/kg bw. Two animals were slaughtered at each time point (0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h) and serum concentrations determined. Peak serum concentrations were 1.92–2.06 µg/ml at 2–3 h, declining to 0.2–0.4 µg/ml at 6–8 h post-treatment. Highest concentrations occurred in the alimentary tract. Concentrations persisted throughout the small intestine and colon for at least 8 h. Urine concentrations ranged from 6 µg/ml at 30 minutes to a peak concentration of 160 µg/ at 4 h. Amoxicillin concentrations were above 50 µg/ml from 1–12 h post-treatment (Palmer, 1975b; Palmer, Bywater and Francis, 1977).

Six calves were treated with an i.m. injection of amoxicillin at 7 mg/kg bw. Serum samples were collected at 0.25, 0.5, 1, 2, 3, 4 and 6 h post-treatment. Highest residues were in body fluids, bile and urine. Mean peak serum concentrations were 3.5–3.6 µg/ml at 1–2 h post treatment. High concentrations persisted in the small intestine for prolonged periods (Palmer, 1975c).

Sixteen pre-ruminating calves received an amoxicillin oral dose of 7 mg/kg bw administered with an oral doser using a 50 mg/ml formulated concentration. Two calves were slaughtered at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h post dose. Peak serum concentrations of 0.7–1.6 µg/ml were found at 4 h and declined to 0.3–0.4 µg/ml at 8 h post-treatment. High amoxicillin concentrations persisted in the small intestine for prolonged periods. Concentrations were approximately ten-fold higher in urine than in serum, although at maximum serum concentration, at approximately 4 h, the ratio was approximately six-fold higher. Peak urine concentration occurred at 8 h. Data indicate that only a small proportion of the dose is absorbed and distributed throughout the tissues when using the oral doser (Palmer, 1975d).

In another pharmacokinetic study in pre-ruminating calves, five animals were treated intravenously with sodium amoxicillin or sodium ampicillin at a dose of 7 mg/kg bw. Blood samples were collected from 15 min to 8 h and assayed using a microbiological method. Results were best fitted by a bi-exponential curve and a two compartmental model. The total volume of distribution was the same for amoxicillin or ampicillin (96%). The serum half-life for the terminal phase for amoxicillin (91 ± 5 min) was longer than for ampicillin (73 ± 7 min) (Palmer, 1976).

Pigs

Several pharmacokinetic studies were conducted in pigs in which animals were treated with amoxicillin by different routes of administration: intravenous (i.v.), i.m. or oral. After i.v. administration, amoxicillin is rapidly distributed and eliminated, as suggested by the low values for volume of distribution at steady-state (VD_{SS}) and its low mean residence times (MRT). Different absolute bio-availability percentages were calculated after oral administration, ranging from 11 to 50%, depending on the formulation type and administration under fed or fasting conditions.

A GLP-compliant comparative cross-over trial was performed in pigs treated with amoxicillin by i.v., i.m. and oral routes in order to investigate the bio-availability of various drug formulations, including: a sodium salt for reconstitution in water and administered intravenously, a trihydrate salt in an oil base administered intramuscularly to produce a conventional duration of plasma concentrations; a trihydrate salt in oil base administered intramuscularly to product a prolonged duration of plasma concentrations; and a trihydrate powder for oral administration as a solution. The concentrations of amoxicillin in plasma were measured by HPLC-Fluorescence and its pharmacokinetic variables were assessed for the individual pigs, using non-compartmental methods. Following i.v. administration (8.6 mg/kg bw), amoxicillin was rapidly eliminated with a MRT of 1.4 h. After i.m. administration of the conventional formulation (14.7 mg/kg bw), the plasma amoxicillin concentration peaked at 2 h at

5.1 µg/ml and the bio-availability was approximately 83%. However, after i.m. administration of the long-acting formulation of amoxicillin, drug bio-availability was calculated to be 111%. In contrast, absorption of amoxicillin after oral administration was slow and incomplete, especially in fed pigs (Agerso and Friis, 1998). The C_{max} value of 1.6 mg/ml was observed in fasted pigs after 1.9 h), while a lower peak concentration of 0.8 mg/ml was reached after 3.6 h in fed pigs (Agerso and Friis, 1998). Oral bio-availability was only 31% in fasted animals and 28% in fed animals. The reported differences in bio-availability, C_{max} and the time to maximum serum concentration (t_{max}) were not statistically significant. A comparative overview of the pharmacokinetics of amoxicillin in pigs after i.v. and i.m. administration is presented in Table 1.3 (Schwarz *et al.*, 2008).

In agreement with these studies are those performed by Morthorst (2002) that also suggested that the oral bio-availability of amoxicillin is considerably reduced by interaction with feed. After a single oral dose administered in 200 ml drinking water with a 20 mg/kg bw dose of amoxicillin by intra-gastric administration to fasted pigs, the curve depicting the course of amoxicillin concentrations in plasma had an ascending and descending profile with the highest concentration achieved 30 min following amoxicillin administration, with C_{max} and bio-availability of approximately 21.55 mg/ml and 91%, respectively. These two pharmacokinetic parameters are considerably higher in comparison with those attained when amoxicillin was administered with feed.

Table 1.3. Comparative description of important pharmacokinetic parameters in pigs after i.v. or i.m. administrations of different formulations of amoxicillin at different doses

	i.v. administration				
	AUC (mg/h/L)	**VD_{SS} (L/kg)**	**MRT (h)**	**CL_B (L/h/kg)**	
Agerso and Friis, 1998. 8.6 mg/kg, Trial 1	23.5 ± 3.7	0.55 ± 0.05	1.5 ± 0.20	0.37 ± 0.06	
Agerso and Friis, 1998. 8.6 mg/kg, Trial 2	17.0 ± 3.4	0.63 ± 0.17	1.2 ± 0.20	0.52 ± 0.10	
Hernandez *et al.,* 2005. 15 mg/kg	4084 ± 1011 (µg/min/ml)	0.81	1.5 ± 0.42	3.9 ± 1.2 (ml/min/kg)	
Martinez-Larranaga *et al.*, 2004. 20 mg/kg	67.11 ± 4.19	1.07 ± 0.08	3.54 ± 0.43	0.30 ± 0.02	
Morthorst, 2002. 20 mg/kg	23.6 ± 2.44	ND	ND	ND	
Reyns *et al.,* 2009. 20 mg/kg	26.17 ± 4.79	0.42 ± 0.12	0.53 ± 0.06	0.78 ± 0.14	
	i.m. administration				
	t_{max} (h)	**C_{max} (µg/ml)**	**AUC (mg/h/L)**	**MRT (h)**	**Bio-availability**
Agerso and Friis, 1998. 14.7 mg/kg	2.0 ± 0.7	5.1 ± 0.8	33.1 ± 3.9	8.8 ± 2.6	0.82 ± 0.08
Morthorst, 2002. 20 mg/kg	1.21 ± 0.73	8.54 ± 3.4	27.8 ± 10.4	ND	1.18
Tanigawa and Sawada, 2003. 7.5 mg/kg	ND	1.12 ± 0.45	21.0 ± 12.0	ND	ND
Agerso and Friis, 1998. 14.1 mg/kg, LA *	1.3 ± 0.5	1.7 ± 1.0	47.6 ± 7.0	66.8 ± 26.2	1.26 ± 0.24
Tanigawa and Sawada, 2003. 15 mg/kg *	ND	2.81 ± 0.48	42.9 ± 9.93	ND	ND

NOTES: * = Formulation with aluminium stearate, long-acting formulation. ND = not detected.

Sheep and goats

The disposition of amoxicillin was studied after i.v. administration of 20 mg/kg bw single doses to 10 lactating goats. Blood samples were collected at 0, 0.05, 0.10, 0.15, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 5, 7 and 9 h post-dosing (Escudero, Carceles and Vicente, 1996). The plasma concentration-time data were analysed by compartmental pharmacokinetics and non-compartmental methods. The results are depicted in Table 1.4. The disposition curves for both were best described by a bi-exponential equation (two-compartment open model). The study demonstrated that amoxicillin is rapidly distributed and slowly eliminated. Additionally, the half-lives and body clearances of amoxicillin and clavulanic acid did not differ significantly when administered alone or in combination.

Table 1.4. Pharmacokinetic parameters of amoxicillin after i.v. administration to goats at 20mg/kg bw

Pharmacokinetic parameter	Mean ± SD
AUC (mg/h/L)	163.18 ± 22.15
MRT (h)	1.47 ± 0.19
CL (L/h/kg)	0.12 ± 0.01
VD_{SS} (L/kg)	0.16 ± 0.02

A study using 10 sheep was designed to examine the pharmacokinetics of amoxicillin sodium salt after i.v. and i.m. administration and after i.m. administration of a suspension of the trihydrate salt to sheep. Animals were allocated to sequences of treatment according to a crossover design: a single dose of 10 mg/kg of a solution of sodium amoxicillin for i.v. and i.m. administration and the same dose of a suspension of trihydrate amoxicillin for i.m. administration. Sampling was done before treatment and 1, 5, 10, 15, 30 and 45 min and 1, 1.5, 2, 2.5 and 3 h after the i.m. administration; before treatment and 5, 10, 15, 30 and 45 min and 1, 1.5, 2, 3, 4 and 5 h after the i.m. administration of sodium amoxicillin; and before treatment and 15, 30 and 45 min and 1, 1.5, 2, 4, 6, 8, 10 and 12 h after the i.m. administration of amoxicillin trihydrate. Amoxicillin disposition was best described by a bi-exponential equation. The results are summarized in Table 1.5. The rapid disposition constant (α) of 14.36 ± 5.30/h and the slow disposition constant (β) of 1.92 ± 0.48/h indicate a rapid distribution and elimination of the drug following i.v. administration. Following i.m. administration of sodium amoxicillin, a greater antibiotic persistence was observed in plasma in comparison with i.v. administration. A slower disappearance was observed with the trihydrate amoxicillin suspension relative to the sodium amoxicillin administered by the same route. The absolute bio-availability of trihydrate amoxicillin suspension was 73%, which was similar to that obtained with sodium amoxicillin (69%) (Fernandez *et al.*, 2007).

Table 1.5. Pharmacokinetic parameters of amoxicillin in sheep after i.v. and i.m. administration at a dose of 10 mg/kg bw

i.v. administration		**i.m. administration**			
Sodium amoxicillin		**Sodium amoxicillin**		**Trihydrate amoxicillin**	
Parameter	**Mean ± SD**	**Parameter**	**Mean ± SD**	**Parameter**	**Mean ± SD**
$AUC_{0-\infty}$ (μg/h/L)	21.83 ± 8.00	$AUC_{0-\infty}$ (μg/h/L)	15.05 ± 1.82	$AUC_{0-\infty}$ (μg/h/L)	15.40 ± 1.05
MRT (h)	0.48 ± 0.15	MRT (h)	1.07 ± 0.30	MRT (h)	8.57 ± 2.78
α (h^{-1})	14.36 ± 5.30	C_{max} (μg/L)	13.42 ± 5.36	C_{max} (μg/L)	2.48 ± 0.54
β (h^{-1})	1.92 ± 0.48	t_{max}(h)	0.36 ± 0.21	t_{max}(h)	0.98 ± 0.15
$t_{1/2}$ (h)	0.38 ± 0.09	$t_{1/2}$ (h)	0.55 ± 0.15		

Two comparative pharmacokinetic studies were performed to investigate whether inter-species differences in amoxicillin disposition could exist after drug i.v. administration (single dose of 10 mg/kg) to sheep and goats (Craigmill, Pass and Wetzlich, 1992.; Elsheikh *et al.*, 1999). Results are

summarized in Tables 1.6 and 1.7, respectively. Both studies revealed no significant differences between any of the pharmacokinetic parameters measured in sheep and goats.

Table 1.6. Pharmacokinetic parameters of amoxicillin in sheep and goats after i.v. administration of a single amoxicillin dose at 10 mg/kg bw (Craigmill, Pass and Wetzlich, 1992)

Pharmacokinetic parameter	Sheep (n=6) Mean ± SD	Goats (n=5) Mean ± SD
AUC (µg/min/ml)	1004 ± 111	895 ± 129
CL (ml/min/kg)	10.1 ± 1.1	11.41 ± 1.61
VD (ml/kg)	667 ± 106	953 ± 350
VD_{SS} (ml/kg)	220 ± 20	470 ± 259
$t_{1/2}\alpha$ (min)	11. ± 7-	10. ± 5-
$t_{1/2}\beta$ (min)	46. ± 3	66 ± .9-

Table 1.7. Pharmacokinetic parameters of amoxicillin in sheep and goats after i.v. administration of single amoxicillin dose at 10 mg/kg bw (Elsheikh *et al.,* 1999)

Pharmacokinetic parameter	Sheep (n=5) Mean ± SD	Goats (n=5) Mean ± SD
AUC (µg.min/ml)	1603.47 ± 233.03	1832.73 ± 289.68
CL (ml/min.kg)	6.34 ± 1.03	5.42 ± 0.78
VD_{SS} (L/kg)	0.46 ± 0.08	0.39 ± 0.06
$t_{1/2l}$ (min) (harmonic mean)	8.38 ± 1.39	6.43 ± 0.85
$t_{1/2z}$ (min) (harmonic mean)	76.01 ± 10.58	61.22 ± 12.79

No differences between pharmacokinetic parameters obtained after i.m. administration at 10 mg/kg to animals from either species were found (Table 1.8). While plasma drug concentrations versus time after i.v. administration were better fitted to a two-compartmental model, plasma drug concentrations obtained after i.m. administration were better fitted to a one-compartmental model with first order absorption and elimination rates. The bio-availability of amoxicillin, more than 90% for goats and sheep, indicated almost complete absorption of amoxicillin when it was intramuscularly administered.

Table 1.8. Pharmacokinetic parameters of amoxicillin in sheep and goats after i.m. administration of single amoxicillin dose at 10 mg/kg bw (Elsheikh *et al.*, 1999)

Pharmacokinetic Parameter	Sheep (n=5) Mean ± SD	Goats (n=5) Mean ± SD
C_{max} (µg/ml)	9.47 ± 1.33	11.03 ± 0.97
T_{max} (h)	54.1 ± 7.6	50.9 ± 6.4
MRT (h)	128.8 ± 9.4	121.9 ± 14.8
AUC (µg/min/ml)	1512.7 ± 128.8	1685.9 ± 182.0
F	0.95 ± 0.06	0.91 ± 0.09

NOTES: F = Bioavailability

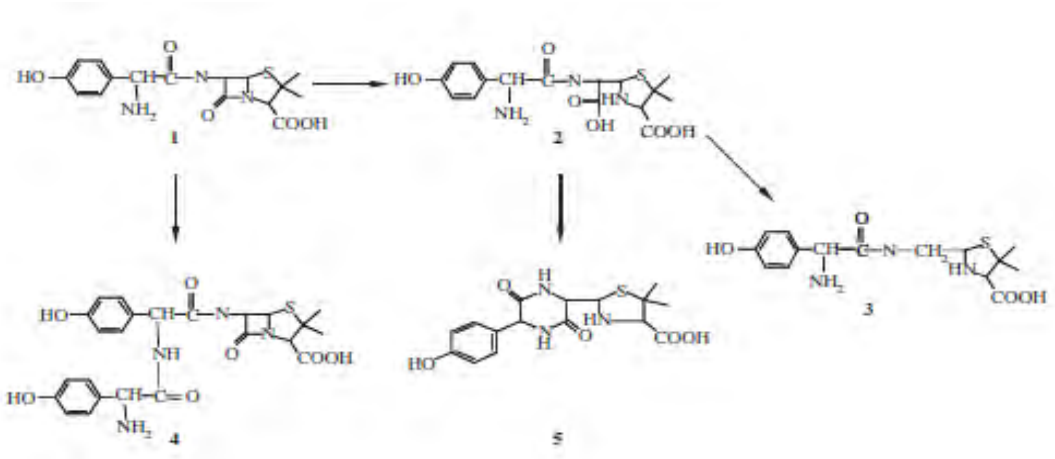


Figure 1.3. Principle metabolic pathway of amoxicillin.
KEY: (1) Amoxicillin; (2) Amoxicilloic acid; (3) Amoxilloic acid; (4) 4-Hydroxyphenylglycyl amoxicillin; (5) Amoxicillin piperazine-2',5'-dione.

Metabolism

The two major metabolites of amoxicillin are amoxicilloic acid and amoxicillin piperazine-2,5-dione (diketopiperazine). These metabolites have lost the antibacterial activity of the parent component, but the amoxicilloic acid could have potential allergic properties (Reyns *et al.*, 2008a). Figure 1.3 shows the degradation of amoxicillin to its major metabolites, amoxicilloic acid and amoxicillin piperazine-2',5'-dione, and two minor inactive metabolites, after the addition of 1 ml 0.1 M HCl solution to 1 ml of amoxicillin solution (25 mg/ml in Dimethyl sulphoxide [DMSO]) (Nagele and Moritz, 2005).

Metabolism in laboratory animals

Rats

In healthy adult male Wistar rats orally dosed with amoxicillin (once at 15 or 60 mg/kg bw), amoxicillin was not substantially metabolized, as 60–75% was excreted unchanged in urine within 24 h. Some amoxicillin was transformed to amoxicilloic acid and amoxicillin diketopiperazine-2,5-dione (Fujiwara *et al.*, 2011).

Metabolism in food-producing animals

Pigs

In pigs, amoxicillin is rapidly metabolized to amoxicilloic acid and amoxicillin diketopiperazine after i.v., oral and s.c. administrations, as shown in Table 1.9 and Figure 1.4 (Reyns *et al.,* 2009). The absence of a hepatic first-pass effect of amoxicillin in pigs was demonstrated, and pre-systemic degradation of amoxicillin in the gut and liver and hydrolysis of amoxicillin by blood enzymes do not seem to be responsible for bio-transformation or for the low oral bio-availability.

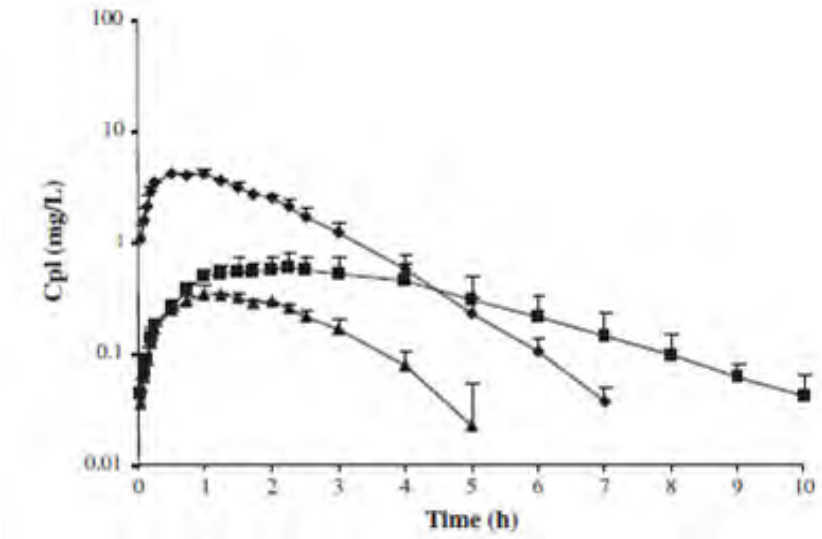


Figure 1.4. Plasma concentrations of amoxicillin, amoxicilloic acid and amoxicillin diketopiperazine in jugular venous plasma after single s.c. administration of amoxicillin at 20 mg/kg bw in pigs.
NOTES: Amoxicillin (diamond curve), amoxicilloic acid (square curve) and amoxicillin diketopiperazine (triangle curve). LOQ = 50 µg/kg (n=2; mean ± SD).

In another non-GLP study, incurred tissue (liver, muscle, kidney and fat) samples were obtained from pigs that received amoxicillin via the drinking water (De Baere *et al.*, 2002). The pigs were slaughtered after cessation of medication at 12, 36, 60 and 108 h and the tissue samples analysed. The amoxicillin concentrations were >10 times above 50 µg/kg in kidney but at or below 50 µg/kg in all other tissues at 12 h after cessation of medication. At 36 h, nearly all tissues contained no detectable amoxicillin. The amoxicilloic acid metabolite, however, persisted much longer in kidney and liver tissues at concentrations much higher than 50 µg/kg. In muscle and fat tissues, the presence of these metabolites was negligible. The amoxicillin diketopiperazine metabolite was found in low concentrations and had nearly disappeared in all tissues within 36 h (<LOQ). Results are presented in Table 1.9.

Table 1.9. Pharmacokinetic parameters of amoxicilloic acid and amoxicillin diketopiperazine in portal and jugular venous plasma after single i.v. or oral administration of amamoxicillin at a dose of 20 mg/kg bw in pigs

Pharmacokinetic parameter	Amoxicilloic acid		Amoxicillin diketopiperazine	
	Portal vein	Jugular vein	Portal vein	Jugular vein
i.v. route				
$AUC_{0-\infty}$ (mg/h/L)	7.82 ± 2.14	8.22 ± 2.01	1.13 ± 0.09	1.26 ± 0.08
$t_{1/2(el)}$ (h)	1.94 ± 0.21	1.85 ± 0.29	0.41 ± 0.04	0.45 ± 0.02
Oral route				
$AUC_{0-\infty}$(mg/h/L)	8.01 ± 2.01	7.55 ± 2.44	0.37 ± 0.11	0.31 ± 0.11
$t_{1/2(el)}$ (h)	3.30 ± 2.70	2.07 ± 0.46	0.88 ± 0.62	0.84 ± 0.66
C_{max} (mg/L)	2.10 ± 0.28	1.83 ± 0.72	0.15 ± 0.75	0.15 ± 0.02
t_{max} (h)	2.60 ± 0.98	2.45 ± 0.40	2.13 ± 0.40	2.13 ± 0.60

TISSUE RESIDUE DEPLETION STUDIES

Radiolabelled residue depletion studies

There were no amoxicillin radiolabel residue depletion studies in cattle, pigs or sheep for evaluation. The only microbiological active residue is the parent drug using microbiological agar gel assays with either *Sarcina lutea* or *Bacillus subtilis* as the test organism (Acred *et al.,* 1970).

Residue depletion studies with unlabelled drug

Pre-ruminating calves

Eighteen 1–2-week-old calves weighing 34–45.5 kg (mean body weight = 39.7 kg) were treated orally with 500 mg amoxicillin soluble powder twice daily for five days in milk replacer. All the animals, regardless of weight, were treated with the same 500 mg dose. Three animals were assigned to each treatment group. Samples of muscle, liver, kidney, fat and blood serum were collected at 1, 3, 5, 7, 9 and 11 days post-treatment. The group slaughtered at day 1 contained animals with the lowest mean body weight, 35.9 kg; animals slaughtered at day 3, 41.4 kg; day 5 slaughter, 38.8 kg; day 7 slaughter, 42.4 kg; day 9 slaughter, 39.7 kg; and day 11 slaughter, 37.3 kg. Results were determined by a microbiological assay and are summarized in Table 1.10 (Keefe, 1976a).

Thirty pre-ruminating calves were treated orally with a 400 mg amoxicillin bolus twice daily for five days. Three animals were sampled in each group at 4 h, 1, 3, 5, 7, 9, 11, 12, 14 and 16 days. Mean body weights for the ten groups of animals were: group 1, 46.3 kg; group 2, 41.7 kg; group 3, 40.6 kg; group 4, 40.5 kg; Group 5, 45.2 kg; group 6, 41.2 kg; group 7, 41.1 kg; group 8, 43.9 kg; group 9, 41.7 kg; and group l0, 47.0 kg. Results are shown in Table 1.11 (Smith *et al.,* 1975a).

Table 1.10. Residue depletion in pre-ruminating calves treated with 500 mg twice daily of soluble powder (mg/kg)

Tissue	Day 1	Day3	Day 5	Day 7	Day 9	Day11
Muscle	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Liver	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	<0.01	<0.01	<0.01	<0.01	0.01	<0.01
Kidney	0.09	<0.01	<0.01	No sample	<0.01	<0.01
	0.12	<0.01	<0.01		<0.01	<0.01
	0.12	<0.01	<0.01		<0.01	<0.01
Fat	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table 1.11. Depletion study in pre-ruminating calves with a 400 mg twice daily bolus (mg/kg)

Tissue	4 h	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 12	Day 14
Muscle	0.01	<0.01	0.02	0.01	0.02	0.01	0.02	<0.01	<0.01
	0.03	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	<0.01	<0.01
	<0.01	<0.01	<0.01	<0.01	0.05	<0.01	0.02	<0.01	<0.01
Liver	0.03	0.02	0.01 0.01	0.01	<0.01	0.06	0.04	<0.01	<0.01
	0.04	0.02	<0.01	0.01	<0.01	0.03	0.04	<0.01	<0.01
	0.01	0.02		0.01	0.02	0.05	0.07	<0.01	<0.01
Kidney	0.16	0.01	<0.01	<0.01	<0.01	0.02	0.01	0.02	<0.01
	0.16	0.11	<0.01	0.03	<0.01	<0.01	0.01	<0.01	<0.01
	0.05	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01
Fat	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	<0.01	<0.01	<0.01	<0.01	0.04	<0.01	<0.01	<0.01	<0.01

Table 1.12. Depletion study in non-ruminating calves dosed with 400 mg twice daily for 5 days with a soluble powder formulation (mg/kg)

Tissue	Day 15	Day 18	Day 20
Muscle	<0.010	<0.010	<0.010
		<0.010	<0.010
		<0.010	<0.010
Liver	<0.010	<0.010	<0.010
		<0.010	<0.010
		<0.010	<0.010
Kidney	<0.010	<0.010	<0.010
		0.210	<0.010
		0.010	<0.010
Fat	<0.016	<0.010	<0.010
		0.244	<0.010
		0.020	<0.010

Twelve pre-ruminating calves were treated with amoxicillin soluble powder at 400 mg twice daily for five days. There were three animals per group, and sampling was done at 15, 18 and 20 days. Mean body weights were not provided. However, calves weighing 36.4–45.5 kg were used in this study. Results are in Table 1.12. Two animals in group one expired prior to slaughter (Keefe, 1976b).

In a residue depletion study in pre-ruminating calves (Keefe, 1976c), 21 animals (36.4–45.5 kg bw) were treated with an amoxicillin suspension by deep i.m. injection using a 250 mg/ml suspension at a dose rate of 17.6 mg/kg bw once a day for seven days. The recommended dose is 400 mg/ml for a 45.5 kg bw, equivalent to 8.8 mg/kg bw. The first six days of dosing was in the right leg and the seventh dosing was in the left leg, and referred to as the injection site for sampling. Animals in groups of three were slaughtered at days 1, 5, 9, 12, 15, 18 and 21 after drug administration. The shoulder was sampled as the non-injection site muscle. The assay organism in this study was *Bacillus stearothermophilus*. Sensitivity of the assay was 0.010 mg/kg. Results are presented in Table 1.13. As the data show, there are some values reported as approximate and a substantial number are non-sampled data points.

Table 1.13. Depletion study in pre-ruminating calves dosed at 17.6 mg/kg bw once a day by i.m. injection (mg/kg)

Tissue	Day 1	Day 5	Day 9	Day 12	Day 15	Day18	Day 21
Injection site muscle	6.4 ~4.5 0.2	0.27 0.19 6.4	~1.2 <0.01 n.s.	<0.01 0.12 ~2.0	<0.01 n.s. n.s.	0.18 n.s. <0.01	<0.01 <0.01 <0.01
Muscle	~0.40 ~0.40 0.31	~0.4 <0.01 <0.01	<0.01 <0.01 n.s.	<0.01 <0.01 <0.01	<0.01 n.s. n.s.	<0.01 n.s <0.01	<0.01 <0.01 <0.01
Liver	~1.2 ~1.2 ~1.2	0.02 0.01 0.02	<0.01 <0.01 n.s.	<0.01 <0.01 <0.01	<0.01 n.s. n.s.	<0.01 n.s <0.01	<0.01 <0.01 <0.01
Kidney	~10 ~10 ~10	0.09 0.03 0.05	0.01 <0.01 n.s.	<0.01 0.02 <0.01	<0.01 n.s. n.s.	<0.01 n.s <0.01	<0.01 <0.01 <0.01
Fat	~0.4 0.2 0.2	<0.01 0.02 <0.01	<0.01 <0.01 n.s.	<0.01 <0.01 <0.01	<0.01 n.s. n.s.	<0.01 n.s <0.01	<0.01 <0.01 <0.01

NOTES: n.s. = not sampled.

Ruminating calves

Thirty-three ruminating calves weighing 159–363.6 kg were treated with amoxicillin (250 mg/ml) by deep muscular injection at a dose of 17.6 mg/kg bw once daily for seven days, with no more than 15 ml administered in one injection site. For the first six days, drug was administered in the right leg. The seventh injection was in the left leg, serving as the injection site for muscle sampling. Three animals were sacrificed at each time point: 3 h, 1, 3, 5, 6, 7, 8, 9, 11, 13 and 15 days. Results are shown in Table 1.14 (Smith et al., 1975a).

In another residue depletion study for ruminating calves, 15 animals (body weights ranging from 136.4 to 204.5 kg) were treated with an amoxicillin trihydrate suspension (250 mg/ml) using deep muscle injection daily at a dose rate of 17.6 mg/kg body weight for seven days. Injection protocol was as described in the previous study with sampling times post treatment at 13, 16, 19, 22 and 25 days. Results are summarized in Table 1.15 (Smith *et al.,* 1976).

Fifteen ruminating calves, weighing 136.4-204.5 kg, were treated with amoxicillin suspension (250 mg/ml) administered by s.c. injection at 17.6 mg/kg bw for seven days. In this study, the injection was in the right side of the neck for six days and the seventh injection in the left side, for measuring the injection site residues. Sampling was at 2, 15, 18, 21 and 25 days. However, the microbial culture from samples taken on days 2, 15 and 18 did not grow, and the 0.01 mg/kg samples did not give a zone of inhibition. Results from all tissue samples collected on days 21 and day 25 were all reported as containing less than 0.01 mg/kg (Smith and Moore, 1976).

Table 1.14. Tissue residues in ruminating calves after i.m. treatment with 17.6 mg/kg bw dose once daily for seven days (mg/kg)

Withdrawal time	Mean b.w.	Injection site	Muscle	Liver	Kidney	Fat
3 hours	228.0 kg	>0.16	>0.16	>0.16	>0.16	>0.16
		>0.16	>0.16	>0.16	>0.16	>0.16
		>0.16	>0.16	>0.16	>0.16	>0.16
1 day	157.6 kg	>0.16	>0.16	>0.16	>0.16	>0.16
		>0.16	0.11	>0.16	>0.16	>0.16
		>0.16	>0.16	>0.16	0.13	>0.16
3 days	159.1 kg	>0.16	0.01	0.13	0.05	0.04
		>0.16	0.02	0.11	0.04	0.02
		>0.16	0.02	0.09	0.03	0.01
5 days	209.0 kg	>0.16	<0.01	>0.16	0.06	<0.01
		0.01	0.01	<0.01	0.02	<0.01
		>0.16	<0.01	0.09	<0.01	<0.01
6 days	213.6 kg	>0.16	<0.01	0.07	0.84	<0.01
		<0.01	<0.01	0.07	0.03	<0.01
		0.03	<0.01	0.11	0.04	0.02
7 days	179.5 kg	>0.16	<0.01	0.12	<0.01	0.01
		<0.01	<0.01	0.06	<0.01	0.01
		<0.01	<0.01	0.11	0.02	0.01
8 days	304.5 kg	0.05	<0.01	0.08	<0.01	<0.01
		>0.16	<0.01	0.11	<0.01	<0.01
		>0.16	<0.01	>0.16	<0.01	<0.01
9 days	333.3 kg	<0.01	<0.01	0.12	<0.01	<0.01
		>0.16	<0.01	>0.16	<0.01	<0.01
		<0.01	<0.01	>0.16	<0.01	<0.01
11 days	152.3 kg	<0.01	<0.01	<0.01	<0.01	<0.01
13 days	142.2 kg	<0.01	<0.01	<0.01	<0.01	<0.01
15 days	134.8 kg	<0.01	<0.01	<0.01	<0.01	<0.01

Table 1.15. Tissue residues in ruminating calves after i.m. treatment with 17.6 mg/kg amoxicillin suspension once daily for seven days (mg/kg)

Withdrawal time (days)	Injection site muscle	Muscle	Liver	Kidney	Fat
13	<0.02	0.03	<0.04	0.01	<0.01
	0.23	0.14	<0.04	0.01	<0.01
	0.07	0.03	<0.04	<0.01	<0.01
16	<0.02	0.04	<0.04	<0.01	<0.01
	<0.02	0.04	<0.04	<0.01	<0.01
	<0.03	0.03	<0.04	<0.01	0.04
19	<0.01	0.01	<0.01	<0.01	0.05
	<0.01	<0.01	<0.01	<0.01	<0.01
22	0.04	0.03	<0.01	0.04	0.15
	0.09	<0.01	<0.01	<0.01	0.06
	<0.01	<0.01	<0.01	<0.01	0.10
25	<0.01	<0.01	<0.01	<0.01	<0.01
	<0.01	<0.01	<0.01	<0.01	<0.01
	<0.01	<0.01	<0.01	<0.01	<0.01

A GLP-compliant residue depletion study was performed with 10 treatment groups of 4 animals each with a single i.m. injection per day for five consecutive days at 24-hour intervals (Connolly, Prough and Lesman, 2006a). The dose administered was ≥7 mg amoxicillin equivalents per kg bw. Upon necropsy, liver, kidneys, muscle, fat, 2nd and 5th injection sites and tissue surrounding the 2nd and 5th injection sites were assayed using a validated method (LOQ = 50 µg/kg). Because the 2nd and

5th injection sites were collected and these injections had been administered three days apart, the final withdrawal time data were generated at 2, 5, 6, 9, 10, 13, 14, 17, 21, 24, 28, 31, 35, 38, 42, 45, 49, 52, 56 and 59 days post 5th dose. The results are presented in Table 1.16. Amoxicillin residues in liver, muscle, kidney and fat fell below 50 µg/kg by 2 days following treatment and were below the method LOQ for all subsequent sampling times. After 28 days, the amoxicillin residues fell below 50 µg/kg at the injection site. For the 42-day injection site sample from one animal the amoxicillin residues were >50 µg/kg.

Table 1.16. Mean amoxicillin residues in cattle (µg/kg) treated with five daily i.m. injections at a dose rate of 7 mg amoxicillin equivalents/kg bw

Group	Days post treatment	Primary injection site	Surrounding injection site	Liver	Muscle	Kidney	Fat
1	2	70 981	22 550	ND	< 10.0	40.9	< LOQ
	5	6 854	< 3 350				
2	6	5 977	< 783	ND	ND	ND	ND
	9	1 264	< 164				
3	10	691	< 94.9	NA	NA	ND	NA
	13	< 315.4	< LOQ				
4	14	522	< 92.4	NA	NA	NA	NA
	17	< 17.7	< 30.8				
5	21	< 55.6	< 106	NA	NA	NA	NA
	24	< 14.0	ND				
6	28	38.4	< 20.6	NA	NA	NA	NA
	31	< 10.5	ND				
7	35	< 14.8	< 10.3	NA	NA	NA	NA
	38	NA	NA				
8	42	< 20.4	< LOQ	NA	NA	NA	NA
	45	NA	NA				
9	49	< LOQ	NA	NA	NA	NA	NA
	52	< LOQ	NA				
10	56	< LOQ	NA	NA	NA	NA	NA
	59	ND	NA				
LOD		0.98	0.98	3.2	0.98	2.10	1.40
LOQ		10	10	25	10	25	10

NOTES: LOD = Limit of detection; LOQ = Limit of quantitation; NA = not analysed; ND not detected.

Lactating dairy cows

Milk samples from a non-GLP compliant study were taken 3, 4, 5 and 6 days after intramammary administration of 5 g of amoxicillin to one cow. Results indicated that 2.7 ng/ml of amoxicillin were present at 3 days post-treatment and that this concentration slowly decreased with time. At 6 days post-treatment, residues of 1.2 ng/ml of amoxicillin persisted in milk (Bruno *et al.,* 2001).

Five lactating dairy cows in at least their 2nd to 6th lactation were selected for the first (Keefe and Kennedy, 1983a) of several studies. Cows were milked out prior to the i.m. administration of amoxicillin trihydrate (250 mg/ml) at 11 mg/kg bw once a day for five days. Sampling of milk began at 12 h post-treatment and continued for eight subsequent milkings. Milk production was recorded. All zero hour milk samples were negative for amoxicillin. Results are summarized in Table 1.17.

The second study (Keefe and Kennedy, 1983b) followed the same protocol, using five lactating dairy cows in their 2nd to 6th lactation. Cows were treated with amoxicillin trihydrate (250 g/ml) at 11 mg/kg bw once a day subcutaneously, with no more than 30 ml per injection site. Milk sampling began at 12 h post-treatment and continued for eight subsequent milkings. Milk production was recorded. Results are summarized in Table 1.18.

Table 1.17. Milk residues following i.m. administration of 11 mg/kg once daily of amoxicillin trihydrate (mg/l) (Keefe and Kennedy, 1983a)

Cow	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h
13	0.02	<0.01	<0.01	<0.01	<0.01	n.s.	<0.01	<0.01
26	0.02	<0.01	<0.01	<0.01	n.s.	<0.01	<0.01	<0.01
28	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
507	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
510	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

NOTES: n.s. = not sampled.

Table 1.18. Milk residues following s.c. administration of 11 mg/kg once daily of amoxicillin trihydrate (mg/l) (Keefe and Kennedy, 1983b)

Cow	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h
86	0.01	0.17	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
93	0.02	<0.01	<0.01	<0.01	n.s.	<0.01	<0.01	<0.01
511[(1)]	0.10	0.07	0.05	0.04	0.02	0.02	0.02	0.02
588	0.03	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
595	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

NOTES: (1) Animal 511 had a positive zero-hour sample which remained positive in the penicillinase-treated sample. All other zero-hour samples were negative.

A study was carried out with six lactating dairy cows treated by i.m. injection with amoxicillin aqueous injectable suspension (250 mg/ml) at a dose rate of 6.6 mg/kg bw (Buswell and Lay, 1974). Although blood samples and milk samples were collected, only the milk residues were reported. Sampling was done at 15, 30, 45 and 60 minutes post-treatment, followed by 1.5, 2, 3, 4, 6, 8 and 24 hour sampling. This study implies that there are very low concentrations in milk even for very short post-treatment periods. Milk residue concentrations are summarized in Table 1.19.

Table 1.19. Milk amoxicillin residues following 6.6 mg/kg bw once daily i.m. administration to lactating cows (mg/l) (Buswell and Lay, 1974)

Milk yield (kg)	mg/l amoxicillin at post-treatment intervals										
	15 min	30 min	45 min	60 min	1½ h	2 h	3 h	4 h	6 h	8 h	24 h

10.9	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.02	0.02	<0.01
8.2	0.02	0.08	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.02	0.02	<0.01
9.1	0.07	0.11	0.02	0.01	<0.01	<0.01	0.03	0.04	0.03	0.05	<0.01
7.3	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.01	<0.01
6.4	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.03	0.07	<0.01

In a study conducted by Barr (1977), five lactating dairy cows were treated with amoxicillin trihydrate (250 mg/ml) by deep i.m. injection at 11 mg/kg bw once a day for five days. Dosing was done following complete milking out of each cow. Sampling began at 12 h post dose and continued for eight milkings. Results are summarized in Table 1.20. Another study was conducted using the same protocol as described above, with treatment by s.c. injection (Keefe, 1976d). Results are shown in Table 1.21.

Table 1.20. Milk residues following i.m. administration of 11 mg/kg once daily of amoxicillin trihydrate (mg/L) (Barr, 1977)

Cow	0 h	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h
108	<0.01	0.83	0.01	0.14	0.17	<0.01	0.15	<0.01	<0.01
129	<0.01	0.04	0.21	0.27	0.07	<0.01	0.15	0.12	<0.01
263	0.20	0.02	0.05	0.19	0.14	0.02	0.26	<0.01	0.01
341	0.18	0.15	0.02	0.10	0.15	0.02	0.14	<0.01	0.96
349	0.11	0.05	0.03	0.14	<0.01	1.57	0.20	0.17	0.79

Table 1.21. Milk residues following s.c. administration of 11 mg/kg once daily of amoxicillin trihydrate (mg/l) (Keefe, 1976d)

Cow	0 h	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h
8	<0.01	0.04	0.06	0.07	0.02	0.05	<0.01	<0.01	<0.01
10	<0.01	0.08	0.60	0.01	0.01	0.01	<0.01	0.09	<0.01
13	0.01	0.07	0.13	<0.01	<0.01	0.16	<0.01	<0.01	<0.01
16	<0.01	0.02	0.01	0.01	0.01	0.01	<0.01	<0.01	<0.01
30	<0.01	0.06	0.22	0.02	0.09	0.15	0.02	<0.01	<0.01

Table 1.22. Amoxicillin concentrations present in milk of a lactating cow treated in all four quarters with amoxicillin trihydrate at 62.5 mg/10 ml per quarter

Hours post-dosing	Amoxicillin in milk (ng/ml)
8	968
24	12.6
32	10.0
48	5.5
56	5.5
72	< LOD

A lactating cow was given amoxicillin trihydrate (62.5 mg/10 ml of in plastet form), infusing one plastet into each quarter of the udder, for a total of 250 mg of drug administered (intra-mammary infusion). Milk samples were collected at 8, 24, 32, 48, 56 and 72 h post-dosing and analysed using a HPLC-UV method with a detection limit of 1.1 ng/ml (Ang *et al.,* 1997). Table 1.22 presents the results.

In a GLP-compliant study, twenty randomly selected dairy cows received five daily i.m. injections of 7 mg amoxicillin equivalents/kg bw at 24-hour intervals (Connolly, Prough and Lesman, 2006b). Pre-dose samples were collected for analytical control purposes from all animals. Raw milk samples were collected at 12-hour intervals for a period of 8 days (16 milkings). The mean amoxicillin concentrations were 9.42 μg/kg at 12 h post-dose, declining to 3.17 μg/kg at 24 h post-dose. Mean residues increased after each of the remaining 4 doses, and subsequently declined rapidly to below 4 μg/kg by 24 h after each respective dose. There was no evidence of bio-accumulation upon repeated dosing. At 12 h following the 5th dose, amoxicillin concentrations averaged 5.84 μg/kg and declined to concentrations below 4 μg/kg at 36 h after the fifth dose, and all samples obtained after 72 h presented concentrations of approximately 0.46 μg/kg. Table 1.23 summarizes the data.

Table 1.23. Mean concentration of amoxicillin residues in milk after treatment of lactating dairy cows with amoxicillin i.m. at 7 mg/kg bw

Sample	Hours post dose 1	Hours post dose 2	Hours post dose 3	Hours post dose 4	Hours post dose 5	Average (μg/kg)
1	0					0.00
2	12					9.42
3	24					3.17
4	36	12				6.61
5	48	24				3.76
6	60	36	12			6.79
7	72	48	24			3.63
8	84	60	36	12		7.03
9	96	72	48	24		3.35
10	108	84	60	36	12	5.84
11	120	96	72	48	24	3.40
12	132	108	84	60	36	2.08
13	144	120	96	72	48	1.32
14	156	132	108	84	60	0.46
15	168	144	120	96	72	0.46
16	180	156	132	108	84	0.46
17	192	168	144	120	96	0.46
18	204	180	156	132	108	0.46
19	216	192	168	144	120	0.46
20	228	204	180	156	132	0.46
21	240	216	192	168	144	0.46
22	252	228	204	180	156	0.46
23	264	240	216	192	168	0.46
24	276	252	228	204	180	0.46
25	288	264	240	216	192	0.46

Amoxicillin trihydrate was administered at an extra-label dosage of 22 mg/kg bw, i.m., once daily to six cows in a non GLP-compliant study. Milk samples were collected at milking prior to drug administration and up to 156 h post-administration. Analyses performed on incurred milk drug concentrations demonstrated that even at the extra-label dosage of 22 mg/kg, no milk residues higher than 10 µg/L were detected beyond the label milk with holding times for amoxicillin (96 h) (Anderson *et al.,* 1996).

Pigs

In a pig tissue residue study, 33 suckling pigs (2.3–3.6 kg) were treated orally by syringe with amoxicillin oil suspension (50 mg/ml) at 22 mg/kg body weight twice daily for five days. Three pigs were slaughtered at 1 hour, 1, 2, 3, 4, 5, 6, 7, 9, 12 and 15 days. (Keefe, 1979). Results are summarized in Table 1.24.

In another pig residue study, nine suckling pigs (2.3–5.9 kg) were treated orally by syringe with amoxicillin oil suspension (50 mg/ml) at 22 mg/kg bw twice daily for five days. Three pigs were slaughtered at 9, 11 and 14 days (Keefe, 1976e). Results are summarized in Table 1.25.

Table 1.24. Residues in suckling pigs following oral administration of 22 mg/kg bw amoxicillin oil suspension twice daily

Tissue	Time post-treatment (days)										
	1 h	1	2	3	4	5	6	7	9	12	15
Muscle	0.04	<0.01	0.03	<0.01	0.02	0.03	<0.01	0.02	<0.01	<0.01	<0.01
	0.04	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01
	0.08	<0.01	<0.01	<0.01	<0.01	0.02	0.01	<0.01	<0.01	<0.01	<0.01
Liver	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01
	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Kidney	>0.16	0.07	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	>0.16	>0.16	<0.01	<0.01	<0.01	0.03	<0.01	<0.01	<0.01	<0.01	<0.01
	>0.16	0.03	<0.01	<0.01	<0.01	0.02	<0.01	<0.01	<0.01	<0.01	<0.01
Fat	0.02	<0.01	<0.01	0.03	<0.01	0.02	0.02	<0.01	<0.01	<0.01	<0.01
	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	<0.01	0.04	<0.01	<0.01	<0.01
	0.01	<0.01	<0.01	0.03	<0.01	0.04	n.s.	<0.01	<0.01	<0.01	<0.01
Skin	>0.16	0.07	0.01	<0.01	<0.01	0.03	<0.01	0.02	<0.01	<0.01	<0.01
	0.06	>0.16	0.04	<0.01	<0.01	0.07	0.01	0.02	<0.01	<0.01	<0.01
	0.12	0.03	0.06	<0.01	<0.01	0.02	<0.01	<0.01	<0.01	<0.01	<0.01

Table 1.25. Residues in suckling pigs following oral administration of amoxicillin oil suspension

Tissue	Time post-treatment		
	9 days	11 days	14 days
Muscle	0.04	<0.01	<0.01
	0.02	0.04	<0.01
	<0.01	0.01	<0.01
Liver	0.02	<0.01	<0.01
	<0.01	0.02	<0.01
	<0.01	0.01	<0.01
Kidney	0.02	<0.01	<0.01
	<0.01	<0.01	<0.01
	<0.01	<0.01	<0.01
Fat + Skin	>0.16	<0.01	<0.01
	<0.05	0.01	<0.01
	<0.05	0.01	<0.01

A GLP-compliant study was conducted to evaluate residue depletion of amoxicillin in tissues of pigs (Adam and Roberts, 2008). Eleven groups (4 animals each) of healthy pigs were subjected to

either no treatment or a single i.m. injection per day for five consecutive days at 24 h intervals. The dose administered was 7 mg amoxicillin-equivalent/kg bw as determined from pre-treatment weighing. Animals were slaughtered 2, 6, 10, 14, 21, 28, 35, 42, 49, 56 and 63 days post 5th injection. Upon necropsy, liver, kidneys, muscle, fat, 4th and 5th injection sites and tissue surrounding the 4th and 5th injection sites were assayed using a validated method. Because the 4th and 5th injection sites were collected and these injections were administered one day apart, the final withdrawal time data were generated at 2, 3, 6, 7, 10, 11, 14, 15, 21, 22, 28, 29, 35, 36, 42, 43, 49, 50, 56, 57, 63 and 64 days post 5th dose. Injection site residues depleted rapidly at the early withdrawal times from a group mean concentration of 11 344 μg/kg at 3 days withdrawal, to less than 180 μg/kg at 11 days withdrawal. Mean residues as well as residues in all individual animals were <LOQ (25 μg/kg) at withdrawal times ≥35 days post-treatment. Mean amoxicillin residues in liver, muscle, kidney and fat fell below 50 μg/kg at 2 days following treatment and were below 50 μg/kg for all subsequent sampling times. Results are summarized in Table 1.26.

Table 1.26. Mean amoxicillin residues (μg/kg) in pigs treated with five daily i.m. injections of 7 mg amoxicillin/kg bw

Group	Days post last treatment	Primary Injection Site	Surrounding Injection Site	Liver	Muscle	Kidney	Fat
1	2	2782	191	ND	ND	< 45.3	ND
	3	11344	4.67				
2	6	1595	252	ND	ND	< LOQ	ND
	7	531	2.1				
3	10	431	215	ND	ND	< LOQ	ND
	11	<180	143				
4	14	438	36.2	ND	< LOQ	< LOQ	< LOQ
	15	313	21.9				
5	21	< 121	34.1	ND	< LOQ	< LOQ	ND
	22	< 44.1	35.8				
6	28	< 47.9	3.60	NA	NA	NA	NA
	29	< 27.0	13.1				
7	35	< LOQ	24.8	NA	NA	NA	NA
	36	< LOQ	0.00				
8	42	< LOQ	0.40	NA	NA	NA	NA
	43	< LOQ	0.00				
9-11	49-64	NA	NA	NA	NA	NA	NA
LOD	2.19	2.19	5.75	2.19	1.68	3.84	
LOQ	25	25	25	25	25	25	

NOTES: NA = not analysed; ND = not detected; LOQ = Limit of quantitation; LOD = Limit of detection.

A non-GLP residue depletion study was conducted in Belgian Landrace stress-negative pigs. Twenty animals received an i.v. bolus of amoxicillin at a dosage of 20 mg/kg bw through a catheter in an ear vein. Animals (n=4) were slaughtered at 12, 48, 60, 72 and 84 h post-dosing. Amoxicillin and its major metabolites, amoxicilloic acid and amoxicillin diketopiperazine, were quantified in kidney, liver, fat and muscle tissues. Similarly, 20 animals received the same dose of amoxicillin by oral administration through a stomach tube. Samples were collected at the same time points (Reyns *et al.,* 2008a). Table 1.27 summarizes the data obtained. Twelve hours after both oral and i.v. administration, amoxicillin concentrations in kidney samples were relatively high, but decreased rapidly, and 36–48 h after treatment, amoxicillin concentrations were below the LOQ of 25 μg/kg in all tissue samples. The amoxicilloic acid metabolite remained much longer in kidney tissue and also in liver, consistent with

other *in vivo* residue depletion tissue studies in pigs (De Baere *et al.,* 2002). The prolonged presence of amoxicilloic acid in the present study leads to a question regarding the risk assessment for amoxicillin because allergic reactions in humans could be a concern in relation to its metabolites.

Table 1.27. Mean tissue concentrations (ng/g) (and Standard Deviations) of amoxicillin (AMO), amoxicilloic acid (AMA) and amoxicillin diketopiperazine (DIKETO) in pig tissue after i.v. and oral administration of amoxicillin at 20 mg/kg bw

		Time and route of administration							
Tissue	**Chemical**	**12 h**		**48 h**		**60 h**		**72 h**	**84 h**
		oral	**i.v.**	**oral**	**i.v.**	**oral**	**i.v.**		
Kidney	AMO	618 (359)	915 (148)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	AMA	10 3132 [(1)] (3 096)	5 575 [(1)] (744)	205 (115)	100 (79)	213 (115)	120 (40)	<LOD	<LOD
	DIKETO	88 (61)	47 (23)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Liver	AMO	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	AMA	1 379 [(2)] (201)	546 [(2)] (198)	35 (14)	<LOQ	42 (24)	<LOQ	<LOD	<LOD
	DIKETO	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Fat	AMO	<LOQ	39 (20)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	AMA	127 (68)	118 (66)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	DIKETO	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Muscle	AMO	<LOQ	35 (18)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	AMA	30 (17)	32 (22)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	DIKETO	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

NOTES: LOD = 1.7, 7.1 and 2.0 µg/kg for AMO, AMA and DIKETO, respectively, in pig kidney; 3.5, 14.2 and 1.6 µg/kg for AMO, AMA and DIKETO, respectively, in liver; 1.5, 11.1 and 0.9 µg/kg for AMO, AMA and DIKETO, respectively, in muscle; and 1.7, 10.6 and 0.8 for AMO, AMA and DIKETO, respectively, in fat. LOQ at least 25 µg/kg for all components in all tissue matrices. (1) Significant at $P = 0.025$. (2) Significant at $P = 0.001$.

Martínez-Larrañaga and co-workers (2004) also performed a study in twelve pigs treated with daily oral doses of 20 mg/kg amoxicillin for five days. The mean concentration (n=4) of amoxicillin in the pig kidneys six days after the last dose was 21.4 µg/kg, and in the liver it was 12.32 µg/kg, but no amoxicillin could be detected in fat or muscle.

Sheep

A GLP-compliant tissue residue depletion study was conducted in 38 crossbred sheep (49–69 kg) randomized into 9 groups of 4 (2 rams + 2 ewes) with one male and one female acting as controls. Each treated sheep received a single i.m. injection per day for five consecutive days at a nominal rate of 7 mg amoxicillin/kg bw (Adam and Roberts, 2007a). Animals were slaughtered at withdrawal times of 2, 6, 10, 14, 21, 28, 35, 42, 49, 56 and 63 days post 5th injection, while tissues (liver, kidneys, muscle, fat) were harvested at 2, 3, 6, 7, 10, 11, 14, 15, 21, 22, 28, 29, 35, 36, 42, 43, 49, 50, 56, 57, 63 and 64 days post 5th dose. All samples were assayed according to a validated method. Amoxicillin concentrations at the injection site depleted from 5, 736 µg/kg at 48 h following the final dose administration to less than 50 µg/kg after 28 days withdrawal. At 64 days, mean residues were at the LOQ (25.6 µg/kg), with one animal having residues <LOD. However, 1 of the 4 animals in this group had an amoxicillin concentration of 60.3 µg/kg. Residues of amoxicillin in liver, kidney, muscle and fat depleted rapidly and 48 h post-dosing all amoxicillin concentrations were lower than 50 µg/kg. Mean amoxicillin residues at the injection site are depicted in Table 1.28.

Table 1.28. Summary of injection site residue data from sheep treated with five daily i.m. injections of amoxicillin at 7 mg/kg bw

Withdrawal time (days)	Mean amoxicillin residues (µg/kg)	Number of animals with >50 µg/kg	Maximum Individual Concentration (µg/kg)
2	5 736	4 of 4	12 700
3	1 558	4 of 4	2 640
6	1 129	4 of 4	2 073
7	813	4 of 4	1 500
10	667	4 of 4	833
11	819	4 of 4	1 918
14	347	4 of 4	916
15	347	4 of 4	660
21	70.7	2 of 4	198
22	58.0	2 of 4	110
28	41.9	1 of 4	84.3
29	28.1	0 of 4	35.3
35	45.4	1 of 4	95.7
36	31.7	1 of 4	72.7
42	31.4	0 of 4	42.5
43	30.8	0 of 4	38.5
49	< LOQ	0 of 4	28.6
50	71.7	1 of 4	142
56	< LOQ	0 of 4	25.1
57	< LOQ	0 of 4	34.2
63	< LOQ	0 of 4	26.0
64	25.6	1 of 4	60.3

Lactating dairy sheep

A GLP-compliant study was performed in 20 lactating dairy sheep that were treated five times intramuscularly with 7 mg amoxicillin-equivalent/kg bw at 24 h (Adam and Roberts, 2006). Raw milk samples were collected at 12-hourly intervals for a period of 10 days (20 milkings). Amoxicillin mean milk residues increased from 23.1 µg/kg at 12 h following the first dose, to 33.0 µg/kg 12 h following the second dose. These mean concentrations were maintained following doses 3 to 5. The mean values obtained for milking samples after the 5th injection are depicted in Table 1.29.

Table 1.29. Amoxicillin residues in milk from sheep administered five consecutive daily i.m. injections of 7 mg/kg bw

	Hours after 5th dose									
	12	**24**	**36**	**48**	**60**	**72**	**84**	**96**	**108**	**120**
Mean concentration (µg/kg)	33.2	17.1	8.68	4.87	2.76	2.33	2.26	2.08	2.09	2.09

The overall results indicate that there was no tendency for bio-accumulation of residues in milk upon repeated dosing. Amoxicillin milk residues declined steadily following cessation of dosing and mean concentrations were <4 µg/kg at 60 h following the final dose administration.

In another study, a solution of 35 mg of clavulanic acid (as the potassium salt) and 140 mg of amoxicillin (amoxicillin trihydrate) per ml was administered to ten sheep. One syringe per udder-half was infused at five consecutive milkings. All animals also received two i.m. infusions at 24 h

intervals. In each animal the first milk sample was taken immediately after the final antibiotic treatment and the subsequent samples were taken at 24 h intervals for 8 days (192 h). As shown in Table 1.30, amoxicillin residues in milk exceeding 4 μg/kg concentrations were detected up to 192 h (8 days) after the last treatment, regardless of the applied preparation (mastitis treatment with two commercial products lactating cows) (Pengov and Kirbis, 2009).

Table 1.30. Mean concentration of amoxicillin in sheep milk

Hours post-final infusion	Concentration mean (μg/kg)	Concentration range (μg/kg)
0	64.0	64.0
24	19.1	15.1–30.5
48	10.2	6.1–21.0
72	9.1	5.6–17.9
96	7.0	4.8–13.1
120	5.9	4.0–8.4
144	5.0	3.5–12.0
168	6.0	4.0
192	4.5	0

A similar study was performed in six lactating ewes. Animals were treated by intramammary infusion with a formulation of 200 mg amoxicillin trihydrate, 50 mg potassium clavulanate and 10 mg prednisolone in a quick release base in a total volume of 3 ml. At 120 h post-treatment, the mean amoxicillin concentration was 0.01 (±0.01) μg/ml. By the final sampling (168 h), the mean concentration was 0.0025 (±0.002) μg/ml (Buswell and Barber, 1989).

Lactating dairy goats

Six lactating Saanen goats, routinely milked, received amoxicillin three times over 24 h by intramammary infusion. The highest concentration of amoxicillin in milk was measured 16 h after the final infusion, 83.3 ± 46.1 μg/ml. By 64 h after the final infusion, milk concentrations were 0.06 ± 0.04 μg/ml (Buswell, Knight and Barber, 1989).

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES AND MILK

Single analytical methods for amoxicillin

Several suitably validated single analyte HPLC methods with fluorescence, UV or mass spectrometry detection for the determination of amoxicillin residues in edible tissues of cattle, pig, sheep and goat, as well as for cow and sheep milk, are available. The performance characteristics are described for some of the methods described by Adam and Roberts (2007b), Neeley and Connolly (2004) and Doran and Adam (2005), including selectivity, LOQ, LOD, robustness, precision and accuracy, that were used for some of the pivotal residue depletion studies in cattle, sheep, pig and goat.

An LC-MS/MS method was validated under GLP-compliant conditions and used for the analysis of edible tissue samples and milk in sheep (Doran and Adam, 2005). Samples of control sheep tissue fortified with amoxicillin were extracted using water followed by a liquid-liquid clean-up using dichloromethane. The final extracts were analysed by a validated LC-MS/MS method. The assay LOQ for amoxicillin was 25 μg/kg for liver, kidney, fat and muscle, and 2 μg/kg for milk. The assay LOD for amoxicillin was 3, 5, 2, 10 and 0.14 μg/kg for ovine liver, kidney, muscle, fat and milk, respectively. The linearity of the method was acceptable over the range 25–100 μg/kg for liver, kidney, fat and muscle and over the range 2–8 μg/kg for milk. The intra-day and inter-day accuracy at concentrations corresponding to approximately 25, 50 and 100 μg/kg and the corresponding precision

were acceptable for all analytes at each concentration. The mean recoveries were all between 67–112% with coefficients of variation of 2–29%.

The stability of amoxicillin was assessed in each matrix at room temperature, freeze/thaw, auto-sampler and extended frozen storage conditions. Liver, kidney and muscle samples were stable following storage at room temperature for approx. 4 h. Skin with fat was not stable, indicating that this matrix should be extracted immediately after thawing. Muscle samples were stable following 3 freeze/thaw cycles. Liver, kidney and skin with fat were not stable, indicating that if additional assays are anticipated, the initial bulk samples should be subdivided prior to storage to provide a new sample for each assay occasion. Liver, kidney and skin with fat samples were stable following storage under auto-sampler conditions (about 4°C) for 48 h; muscle samples were stable following storage under auto-sampler conditions (about 4°C) for 72 h. The extended storage stability data indicate that amoxicillin is stable in muscle for 2 months. Amoxicillin showed limited stability in liver and kidney. No incurred residue stability data were generated as part of this validation study. However, incurred stability was assessed as part of tissue residue depletion studies conducted under separate protocols. The standard solutions were stable for 2 weeks when stored at about 4°C.

A validated analytical method that measured amoxicillin residues in cattle tissues and milk was reported in a GLP-compliant study (Neeley and Connolly, 2004). In this method, tissue samples were extracted in water and cleaned up using methylene chloride. Milk samples were separated into phases and purified using solid phase extraction. Aliquots of the final extracts (liver, kidney, muscle, fat, surrounding injection sites and injection sites) were analysed for amoxicillin. The LOQ was 25 µg/kg for amoxicillin in liver and kidney, 10 µg/kg for muscle and fat and 1.0 µg/kg in milk. At the LOQ of 10 µg/kg, the intra-day accuracy for muscle was 81–84%, 91–94% for fat, and 73–80% for milk. The corresponding precision was 6–15% for muscle, 6–11% for fat and 10–12% for milk at the LOQ of 1 µg/kg. The inter-day precision and accuracy data were similar to those obtained for the intra-day assay precision and accuracy. This validated method was considered to be suitable for use as a routine assay procedure for cattle residue monitoring in edible tissues and milk.

An analytical method developed in 1979 (Melilea and Desai, 1979) determined amoxicillin residues in cattle and pig tissues. The method was validated following existing late-1970 criteria using muscle, liver, kidney and skin tissues. The method had the required sensitivity, selectivity and linearity. Other penicillins did not interfere in the selectivity of the assay. Amoxicillin was extracted from cattle and pig tissues and potential interfering substances were removed by precipitation and extraction. Amoxicillin was converted to a fluorescent compound by heating in an acid medium then separated from other constituents by HPLC and measured quantitatively with a fluorescence detector. The selectivity of the method was demonstrated by the analysis of other penicillins such as ampicillin, penicillin G and cloxacillin. When treated as directed in the method, these substances did not exhibit any fluorescent activity corresponding to the retention time of the amoxicillin derivative. The LOD for amoxicillin was 0.01 mg/kg.

A validation study of an analytical method for the determination of amoxicillin in pig liver, kidney, muscle and skin with fat was reported (Adam and Roberts, 2007b). In this method, control pig tissues were fortified with amoxicillin and extracted using water followed by a liquid-liquid cleanup using dichloromethane. The samples were then further cleaned up using a cation exchange column (WCX SPE) and the final extracts were analysed by LC-MS/MS. The chromatographic system was satisfactory in terms of column efficiency, tailing factor, system precision, linearity of detection and system limit of detection. The LOQ for amoxicillin was 25 µg/kg for liver, kidney, muscle and skin with fat, with mean recoveries between 60 and 95% with coefficients of variation of 2–15%. The LOD for amoxicillin was 6, 2, 2 and 4 µg/kg for pig liver, kidney, muscle and skin with fat, respectively. The method was linear over the range 25–100 µg/kg for liver, kidney, muscle and skin with fat. Significant matrix effects were found in some of the matrices.

The stability of amoxicillin was assessed in each matrix at room temperature, freeze-thaw, autosampler and extended frozen storage conditions. Liver, kidney and muscle samples were stable following storage at room temperature for approx. 4 h. Skin with fat was not stable, indicating that this matrix should be extracted immediately after thawing. Muscle samples were stable following 3 freeze-thaw cycles. Liver, kidney and skin with fat were not stable indicating that if additional assays are

anticipated, the initial bulk samples should be subdivided prior to storage to provide a new sample for each assay occasion. Liver, kidney and skin with fat samples were stable following storage under autosampler conditions (about 4°C) for 48 h; muscle samples were stable following storage under autosampler conditions (about 4°C) for 72 h. The extended storage stability data indicate that amoxicillin is stable in muscle for 2 months. Amoxicillin showed limited stability in liver and kidney. No incurred residue stability data were generated as part of this validation study. However, incurred stability was assessed as part of tissue residue depletion studies conducted under separate protocols. The standard solutions were stable for 2 weeks when stored at about 4°C.

The open literature contains numerous suitably validated single analyte methods (Table 1.31), methods that measure residues of amoxicillin and its two major metabolites, amoxicilloic acid and the DIKETO residues (Table 1.32), and multi-analyte methods for the simultaneous determination of amoxicillin and other veterinary drug residues (Table 1.33). Each of these suitably validated methods whose performance parameters have been summarized in Tables 1.31–1.33 can be used to measure amoxicillin residues in food animal production.

A LC-MS/MS method for the confirmation of amoxicillin residues at the LOQ of 50 µg/kg was also validated. The method showed no matrix effect for muscle or fat. However, MS signal suppression of 18% and 25% was evident in liver and kidney, respectively. MS suppression from the milk matrix occurred to a lesser extent (about 16%).

Suitably validated analytical methods with acceptable performance parameters were used to generate depletion studies in pigs, sheep, cattle and cattle milk. However, because the metabolites of amoxicillin also fluoresce under the acidic conditions used to generate the fluorescent derivative for amoxicillin, analytical methods that use fluorescence for detection cannot be used to make regulatory decisions because those methods tend to overestimate the concentration of residual amoxicillin in treated samples.

Table 1.31. Summary of amoxicillin parent compound analytical methods

Method	Species and tissues	LOD / LOQ	Reported validation	Reference
HPLC Fluorescence	Pig and cattle liver, kidney, muscle, fat	LOD=10 µg/kg	Internal validation on existing criteria	Melilea and Desai, 1979.
HPLC Fluorescence	Pig, cattle and chicken muscle	LOQ=5 µg/kg	FDA guidelines	Luo and Ang, 2000.
LC-MS/MS	Bovine muscle	CCα=61.2 µg/kg CCβ=72.4 µg/kg	EU guidelines	Lugoboni *et al.,* 2011.
LC-MS/MS	Chicken liver, kidney, muscle, fat and skin+fat	CCα=51.6-57.0 µg/kg CCβ=72.4 µg/kg	EU guidelines	de Baere *et al.,* 2005.
LC-MS/MS	Pig liver, kidney, muscle, and skin+fat	LOQ=25 µg/kg LOD=1.7-5.8 µg/kg	OECD guidelines	Adam and Roberts, 2007b.
LC-MS/MS	Sheep liver, kidney, muscle and fat	LOQ=25 µg/kg LOD=2.1-9.7 µg/kg	OECD guidelines	Doran and Adam, 2005.
LC-MS/MS	Bovine liver, kidney, muscle and fat	LOQ=25 µg/kg (liver and kidney) and 10 µg/kg (muscle and fat) LOD=0.98-3.2 µg/kg	OECD guidelines	Neeley and Connolly, 2004.
LC-MS/MS	Bovine milk	LOQ=1 µg/kg LOD=0.08 µg/kg	OECD guidelines	Neeley and Connolly, 2004.
LC-MS/MS	Sheep milk	LOQ=2 µg/kg LOD=0.14 µg/kg	OECD guidelines	Doran and Adam, 2005.

NOTES: CCα = Decision limit; CCβ = Detection capability. European Community, 2002.

Table 1.32. Summary of amoxicillin (Amox), amoxicilloic acid (AMA) and amoxicillin diketopiperazine (Diketo) analytical methods

Method	Species and tissues	LOD / LOQ	Reported validation	Reference
LC-MS/MS	Pig liver, kidney, muscle and fat	LOQ = 25 µg/kg LOD Amox = 1.5–3.5 µg/kg LOD AMA = 7.1–14.2 µg/kg LOD Diketo = 0.8–2.7 µg/kg	EU guidelines	Reyns *et al.,* 2008b.
LC-MS/MS	Pig liver, kidney, muscle and fat	LOQ = 25 µg/kg LOD Amox = 2.3–12 µg/kg LOD AMA = 1.1–15 µg/kg LOD Diketo = 0.2–2.4 µg/kg	EU guidelines	De Baere *et al.,* 2002.
UHPLC-MS/MS	Bovine milk	LOQ = 5 ng/ml LOD Amox = 1.0 ng/ml LOD AMA = 1.0 ng/ml LOD Diketo = 0.2 ng/ml	EU guidelines	Liu *et al.,* 2011.
HPLC-UV	Human urine	LOD = 1.0 ng/ml	Internal validation on existing criteria	Haginaka and Wakai, 1987.
LC-MS/MS	Chicken liver and muscle	CCα = 56 µg/kg CCβ = 67 µg/kg	EU guidelines	Freitas *et al.*, in press.

NOTES: CCα = Decision limit; CCβ = Detection capability.

Table 1.33. Summary of amoxicillin multi-residue analytical methods

Method	Drugs and tissues	Amoxicillin LOD/LOQ	Reported validation	Reference
LC-MS/MS	Ampicillin and amoxicillin in bovine muscle, liver, kidney and milk	LOQmilk = 0.8 µg/kg LODmilk = 0.5 µg/kg LOQtissues = 3 µg/kg LODtissues = 2 µg/kg	EU guidelines	Bogialli *et al.,* 2004.
HPLC-UV	Penicillins in pig muscle including amoxicillin	LOD = 20 µg/kg	Internal validation on existing criteria	McGrane, O'Keeffe and Smyth, 1998.
HPLC-UV	Penicillins in pig muscle including amoxicillin	LOQ = 35 µg/kg LOD = 10 µg/kg	EU guidelines	Verdon and Couëdor, 1999.
HPLC-Fluorescence	Penicillins in bovine serum, kidney and liver including amoxicillin	LOD = 0.02 µg/kg	Internal validation on existing criteria	Hong *et al*., 1995.
HPLC-UV	Penicillins in bovine muscle including amoxicillin	LOD = 10 µg/kg	Internal validation on existing criteria	Boison and Keng, 1998.
HPLC-UV	Penicillins in bovine and pig muscle, liver and kidney including amoxicillin	LOD = 10.1–10.5 µg/kg	Internal validation on existing criteria	Sørensen *et al.,* 1999.
HPLC-UV-MS	β-lactam antibiotics in bovine milk including amoxicillin	LOD = 0.2 µg/L	Internal validation on existing criteria	Tyczkowska *et al.*, 1994.
HPLC-UV	Ampicillin and amoxicillin in bovine muscle and liver	LOQmuscle = 50 µg/kg LOQliver = 100 µg/kg	Internal validation on existing criteria	Rose *et al.,* 1997.
LC-MS-MS	β-lactam antibiotics in bovine kidney including amoxicillin	LOD = 10 µg/kg	FDA guidelines	Fagerquist, Lightfield and Lehotay, 2005.

Method	Drugs and tissues	Amoxicillin LOD/LOQ	Reported validation	Reference
LC-MS-MS	Antibiotics in pig, cattle, sheep, deer, horse and reindeer muscle and kidney, including amoxicillin	LOD = 12 µg/kg	EU guidelines	Granelli and Branzell, 2007.
LC-MS/MS	Penicillins and cephalosporins in bovine muscle, kidney and milk, including amoxicillin	Milk CCα = 4.7 µg/kg CCβ = 5.6 µg/kg Muscle CCα = 53.7 µg/kg CCβ = 57.7 µg/kg Kidney CCα = 58.9 µg/kg CCβ = 69.3 µg/kg	EU guidelines	Becker, Zittlau and Petz, 2004.

NOTES: CCα = Decision limit; CCβ = Detection capability.

APPRAISAL

Amoxicillin is an old compound with a long history of use and has not been previously reviewed by the Committee. Amoxicillin is a beta-lactam antibiotic effective against Gram-positive and Gram-negative bacteria. It is widely used in human and veterinary medicine for the treatment and prevention of respiratory, gastrointestinal, urinary and skin bacterial infections. Amoxicillin is used in a variety of food animals including broiler chickens, pigs, goats, sheep, pre-ruminating calves, including veal calves, and cattle. In human medicine, amoxicillin is widely used in combination with clavulanic acid as a β-lactamase inhibitor. In veterinary medicine, amoxicillin is not commonly used in combination with clavulanic acid.

Pharmacokinetic data on amoxicillin are available for a variety of animal species using various routes of administration and product formulations. In general, amoxicillin is rapidly distributed and eliminated. Relative bio-availability is dependent on formulation and route of administration.

Metabolism data also are available for a variety of animal species. Amoxicillin is moderately to rapidly metabolized to amoxicilloic acid and amoxicillin diketopiperazine, the two major identified metabolites. No antibacterial activity is recognized for these metabolites, but amoxicilloic acid could have potential allergic properties.

No amoxicillin radiolabelled residue depletion data were available for evaluation.

Residue depletion data are available for 5, 4, 6 and 9 studies for pre-ruminating calves, ruminating calves, pigs, lactating dairy cows, respectively, and 1 lactating sheep study. In all studies, amoxicillin residues deplete rapidly. Residues in muscle are universally low, irrespective of species, route of administration or product formulation used. Residues may persist in liver and kidney and in milk for hours to weeks following treatment, depending on the product formulation, dose and route of administration. Only one study, in pigs, provided tissue residue data for amoxicillin, amoxicilloic acid and amoxicillin diketopiperazine simultaneously. In this study, amoxicillin depleted rapidly but amoxicilloic acid is just below LOD in kidney (<7.1 µg/kg) and in liver (<14.2 µg/kg) 72 h post-treatment. For many studies in all three species, the sampling time frames are too long to permit a detailed analysis of residue depletion in tissues and, consequently, there are a substantial number of reported findings <LOQ.

Qualitative and quantitative single or multi-residue methods are available to determine residues of amoxicillin, the main microbiologically active residue identified in edible tissues and milk. Additionally, the two metabolites, amoxicilloic acid and amoxicillin diketopiperazine, can be simultaneously determined by some of the LC-MS/MS methods. Most of the methods have been validated according to internationally accepted standards and would be expected to be suitable for use in regulatory programmes.

MAXIMUM RESIDUE LIMITS

In recommending MRLs for amoxicillin, the Committee considered the following factors:

- An ADI of 0–0.7 μg/kg bw was established by the Committee based on a microbiological end-point, equivalent to an upper bound of 42 μg for a 60 kg person.
- Amoxicillin is primarily metabolized to amoxicilloic acid and amoxicillin diketopiperazine, which have no microbiological activity.
- Amoxicillin is the only microbiologically active residue and is suitable as a marker residue.
- Amoxicillin residues are consistently highest in kidney, and kidney is a suitable target tissue.
- Suitable validated routine analytical methods were available for monitoring purposes.
- The MRLs were based on twice the LOQ of 25 μg/kg for amoxicillin in edible tissues (including skin plus fat in pigs) and of 2 μg/kg for amoxicillin in sheep milk.

The Committee recommended MRLs for amoxicillin in cattle, sheep and pig tissues of 50 μg/kg and in cattle and sheep milk of 4 μg/kg, determined as amoxicillin parent compound. The Committee did not calculate an estimated daily intake (EDI) for amoxicillin owing to the small number of quantifiable residue data points. Using the model diet of 300 g muscle, 100 g liver, 50 g kidney, 50 g fat and 1.5 litre of milk with the MRLs recommended above, the theoretical maximum daily intake (TMDI) is 31 μg/person per day, which represents 74% of the upper bound of the ADI.

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Apramycin

First draft prepared by
Adriana Fernandez Suarez, Buenos Aires, Argentina
Richard Ellis, Myrtle Beach, USA,
and
Bruno Le Bizec, Nantes, France

IDENTITY

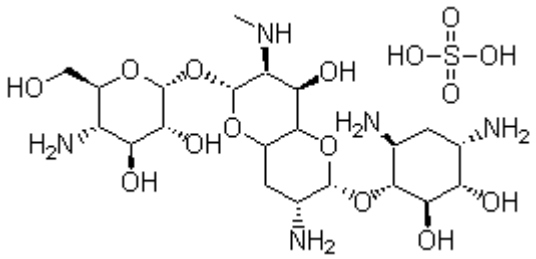
International Non-proprietary names (INN): Apramycin

Synonyms: Laboratory Code EL-820 (EL-857, free base). Compound 47657.
Apralan® is the registered trademark for Elanco® products containing apramycin

IUPAC Names: D-Streptamine, 4-0- [(8R)-2-amino-8-0-(4-amino-4-deoxy-a-D-glucopyranosyl)-2, 3, 7-trideoxy-7-(methylamino)-D-glycero-a-D-allo-octodialdo-1,5: 8, 4-dipyranos-1-yl] -2-deoxy-sulphuric acid salt

Chemical Abstracts Service Number: 41194-16-5

Structural formula of main components:



Molecular formula: $C_{21}H_{41}N_5O_{11}$. 5/2 H_2SO_4

Molecular weight of the salt: 784.8

Molecular weight of the base: 539.6

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient

Apramycin is a broad-spectrum aminocyclitol antibiotic produced by a strain of *Streptomyces tenebrarius*. It is extracted from the fermentation medium as apramycin sulphate with a purity of at least 85%. A microbiological assay is used to determine activity as equivalents of apramycin base. Apramycin is synthesized stereospecifically by *Streptomyces tenebrarius*. It exists as a single enantiomer for which absolute configurations have been determined.

Degree of impurity of Apramycin (produced by Elanco Animal Health)

Total impurities described below are not to exceed 15% (specifications for the release of the product).

Qualitative and quantitative composition of impurities

3 O-Hydroxyapramycin ($C_{21}H_{41}N_5O_{12}$) has a biological spectrum which is very similar to apramycin; microbiological activity is one-half to one-quarter that of apramycin.

Lividimine ($C_{21}H_{41}N_5O_{12}$) is structurally related to apramycin, containing 2-deoxystreptamine and part of the bicyclic portion of the apramycin molecule. Its contribution to the biological activity of apramycin is negligible.

2-Deoxystreptamine ($C_6H_{14}N_2O_3$) is a structural unit of most common aminocyclitol antibiotics, and is biologically inactive.

Compounds A and B are defined on the basis of their thin-layer chromatography. These compounds have not been identified.

Caerulomycin ($C_{12}H_{11}N_3O_3$) is determined as a dipyridyl, as the dipyridyl moiety is a more suitable measurement for the caerulomycin content since a part may be complexed or bound.

Solubility: >300 g/L in water.

RESIDUES IN FOOD AND THEIR EVALUATION

Conditions of use

The Committee evaluated apramycin to establish an ADI and recommend MRLs in cattle, pig and chicken tissues at the request of the 19th session of the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF). The request for MRLs in rabbits was not addressed as there was no approved use of apramycin in rabbits.

Dosage

Indications, recommended doses and duration of treatment for apramycin are presented in Table 2.3, below, in the tissue residue depletion studies section. An injectable form for use in cattle and an oral dose form for use in neonatal lambs, calves and pigs are no longer marketed. Apramycin is not used in human medicine.

Registered uses

Apramycin is registered for use in more than twenty countries in cattle, pigs and chickens. Individual country withdrawal periods for the three species exhibit substantial differences. In cattle, withdrawal periods vary between zero and 42 days. For pigs, the withdrawal periods are 10 to 42 days, and for chickens the withdrawal periods are zero to 14 days.

PHARMACOKINETICS AND METABOLISM

Pharmacokinetics in food-producing animals

Cattle

Calves weighing 38 to 52 kg were administered 20 or 40 mg apramycin activity/kg bw in milk replacer once per day for 5 days (3 male and 3 female calves per dose level) (Van Duyn and Handy, 1978). Following the first, third and fifth doses, blood samples were analysed for apramycin by a microbiological assay up to 24 h post-treatment for the first and third treatments and until 48 h following the fifth treatment. Serum concentrations were broadly similar for each treatment level in both dosages. With the 20 mg/kg bw dose, serum levels peaked at 4 to 6 h after treatment, with mean values from 0.38 to 0.88 µg/ml. Values at 24 h after treatment ranged from undetectable to 0.13 µg/ml. After the fifth and last dose, apramycin serum levels were undetectable 24 h post treatment. After the 40 mg/kg bw dose, serum levels of apramycin were higher than those for the 20 mg/kg treatment. Serum levels peaked at approximately 6 h after treatment, with mean values of 2.49 and 2.40 µg/ml for the first and fifth daily doses, respectively. Mean values 24 h after treatment ranged from 0.15-0.31 µg/ml and duration of activity following the fifth and last dose was between 24 and 36 h.

Male and female Israeli-Friesian calves, 3 to 5 weeks of age, were fed an antibiotic-free milk replacer once a day (Ziv *et al.*, 1985). Apramycin was administered orally as a drench at 20, 30 and 40 mg/kg bw to 10, 9 and 10 calves, respectively. Blood samples were taken 0.5, 1, 2, 3, 4, 6, 8 and 12 h after treatment. Six calves were placed in metabolism cages that facilitated total collection of urine and were administered 20 mg/kg bw apramycin in milk replacer. Maximum serum levels occurred 1 hour after administration. The oral dose of 20 mg/kg bw resulted in mean serum drug levels lower than 0.25 µg/ml and the drug was not detected 12 h post-treatment. After oral dosing of 30 and 40 mg/kg bw, mean peak serum drug concentrations were 1.42 and 1.84 µg/ml, respectively.

Apramycin is very poorly absorbed by the oral route in calves. Even where the area under the serum curves was not reported, it is evident from inspection of the curves that availability of apramycin by the oral route was low. Concentration of apramycin in urine was determined by a microbiological assay. Urine concentrations were very low, i.e. 2.5 µg/ml, but the drug was still detected at 128 h post-treatment. Only 11% of the dose was recovered in urine.

Pigs

A series of studies was conducted in which pigs were administered single doses of apramycin sulphate in aqueous solution by oral gavage and blood levels measured during the next 24 h (Van Duyn and Kline, undated). The pigs ranged in weight from approximately 1.3 to 21 kg and doses ranged from 2.27 to 100 mg apramycin activity per kg bw. Blood levels, determined by a microbiological assay, were low and highly variable. Generally, where measurable levels were found, they peaked between 30 minutes and 4 h after dosing, and declined to below the sensitivity of the assay within 12 to 24 h. In one of the studies, blood levels were compared in 2-day, 4- and 8-week old piglets after dosing with the same (mg/kg bw) apramycin doses. Absorption of apramycin appeared to be most efficient in neonatal pigs, and to decline with age.

Another study (Shang *et al.,* 2004) reported pharmacokinetics of apramycin after single oral or intravenous (i.v.) dosing of pigs with 20 mg apramycin/kg bw. Blood levels fitted a one-compartment open model and a two-compartment open model after oral and i.v. dosing, respectively. For oral treatment, the half-life of elimination was 7.36 h and area under the curve was 4.14, while for i.v. treatment, the half-life of elimination was 3.17 h and area under the curve was 130.62. Oral availability was 3.2%.

One female and two castrated male pigs, weighing approximately 10 kg, were given 25 mg ^{14}C-apramycin/kg bw by oral gavage daily for 5 days (Zornes, Herberg and Donoho, 1979), and 82% to 92% of the dose was recovered in excreta, mostly in faeces. Less than 10% of the dose was found in urine.

Two female and four castrated male pigs, weighing approximately 8 kg, were given 25 mg ^{14}C-apramycin/kg bw by oral gavage daily for 5 days (Herberg *et al.,* 1979). Groups of 1 female and 2 castrated males were slaughtered 4 h or 7 days after the last dose. Balance-excretion data were

collected for the female in the 7-day withdrawal group; 86.9% of the dose was recovered in excreta, of which 81.6% in faeces and 5.3% in urine.

Chickens

Five chickens were administered a single dose of 75 mg/kg apramycin orally into the crop and another five were administered the same dose intramuscularly (i.m.) (Afifi and Ramadan, 1997). Two weeks later, all of the chickens were administered 75 mg/kg i.v. to study drug availability and protein binding. Apramycin was rapidly absorbed when it was administered orally or i.m.; mean times to reach maximum serum concentration after the dose were 0.2 and 0.76 h, respectively. Average half lives for elimination were 1.22 and 2.31 h for the same respective treatments. The maximum concentration reached after oral treatment was only 0.79 µg/ml, compared with 11.6 µg/ml for i.m. treatment, and the respective areas under the curve were 0.81 µg/h/ml and 23.18 µg/h/ml. Bio-availability of apramycin was approximately 2% by the oral route and 58% by the i.m. route.

No excreta data were presented for chickens.

Pharmacokinetics in other species

Rats

Adult rats were given single oral or s.c. doses of 4 mg ^{14}C-apramycin (Donoho *et al.*, 1976). Following oral treatment, 99.5% of the dose was recovered in faeces and 0.5% in urine, while 93% was recovered in urine and 7% in faeces after s.c. dosing.

Dogs

Male and female Beagle dogs with initial weights of 7.2 to 14.6 kg were administered apramycin at 0, 25, 50 or 100 mg/kg bw/day for 6 months (Howard *et al.,* 1977). Dogs were dosed orally with apramycin in gelatin capsules. Blood samples, collected from 2 males and 2 females per treatment group on days 1, 64, 119 and 182 of the study, were analysed for apramycin using a microbiological assay. Mean peak serum levels occurred 2 h after dosing and were 2.2, 5.5 and 11.0 µg/ml for the 25, 50 and 100 mg/kg bw treatments, respectively. All samples collected 24 h after dosing contained <0.5 µg/ml. There was no evidence of accumulation or altered plasma concentrations after prolonged treatment. Urine voided over a 24-hour period was collected from 2 males and 2 females per treatment group on test days 1, 64, 119 and 182 and analysed for apramycin using a microbiological assay. The average proportion of the daily dose that was recovered in urine was 4.0% (standard deviation = 2.6%) with a range of 0.3 to 10.5%.

Summary of pharmacokinetic studies

In calves, pigs, chickens, rats and dogs, apramycin is rapidly yet poorly absorbed by the oral route and quickly eliminated. Mean levels in serum are found a few hours after treatment (up to 6 h) and undetectable at times between 24 and 36 h, depending on the study. Reported availability is poor (3.2% in pigs; < 2% in chickens). More than 80% is primarily recovered in excreta in pigs and rats. The main excreta is in faeces for the mentioned species (more than 82% in pigs and 99.5% in rats), with very low concentrations in urine (11% for cattle; less than 10% for pigs, rats and dogs). Binding of apramycin to serum proteins was 26%, similar to the value found for cows, sheep and goats by Ziv *et al.* (1995).

Metabolism in food-producing animals

Cattle

A calf of approximately 40 kg body weight was given 20 mg/kg ^{14}C-apramycin by i.m. injection on two successive days (Donoho *et al.,* 1976). Total radiolabel dose of apramycin for the calf was 18 µCi. The calf was slaughtered 5 days after the last dose, with the bulk of the radioactivity collected by the end of day 2. Kidney contained the most radioactivity (40.9 mg apramycin equivalent/kg), followed by liver (4.7 mg/kg) and muscle (0.3 mg/kg); fat contained no radioactivity above background. Residues determined by the microbiological assay were compared with the radiolabel results, and 71% of the radioactivity in kidney and 62% of radioactivity in liver was un-metabolized apramycin as determined by the microbiological assay. In blood, levels of radioactivity reached a peak at approximately 30

minutes after injection (maximum concentration was approximately 35 mg/kg-equivalents) and declined to near baseline levels by 24 h. Of the total radioactive dose administered, 84% was recovered in excreta by the end of 5 days following the last dose. More than 93% of the excreted radioactivity was found in the urine within 24 h, and microbiological assay indicated that 90–91% the radioactivity was accounted for as apramycin.

Pigs

A 1979 two-part radiolabel study was conducted by Herberg *et al*. (1979) and Zornes, Herberg and Donoho (1979). In the first part, six piglets weighing approximately 8 kg each were given 25 mg ^{14}C-apramycin/kg by oral gavage daily for 5 days (Herberg *et al.,* 1979). One female and two castrated males were slaughtered at 4 h withdrawal and a similar group was terminated at 7 days withdrawal. Edible tissues were assayed for total radioactivity and selected samples were assayed for apramycin. At 4 h withdrawal, all of the radioactivity in kidney samples was characterized as apramycin After 7 days withdrawal, 80% of the radioactivity in one kidney sample was apramycin, while the other samples contained too little residue for analysis. At 4 h withdrawal, liver samples of the two male piglets contained approximately 85% of unchanged apramycin, while the liver from the female contained at least 50% of apramycin. None of the other tissues contained sufficient radioactivity for apramycin characterization.

In the second part of the study, one female and two castrated male piglets, weighing approximately 10 kg, were given 25 mg ^{14}C-apramycin/kg bw by oral gavage daily for 5 days; they were slaughtered 14 days after the last dose (Zornes, Herberg and Donoho, 1979). Approximately 66% of the kidney residue was unchanged apramycin. In the one liver sample that contained enough residue for characterization, approximately 36% of the total residue was apramycin. In both the faeces and the urine collected, radioactivity was predominantly accounted for by unchanged apramycin (75%)

Chickens

Broiler chickens that were approximately 4 weeks old were given ^{14}C-apramycin (500 mg/L) in the drinking water for five days (Zornes, Herberg and Thomson, 1985). Three chickens were slaughtered 6 h, 7, 10 and 14 days after withdrawal from treatment. Samples with sufficient total activity (all kidney samples and the 6-hour liver samples) were analysed for parent apramycin by bio-autography. Apramycin accounted for 80% or more of total activity in all of the samples.

Summary of metabolism studies

In cattle, pigs and chicken, levels of radioactivity are highest in kidney, followed by liver, with very low quantities in muscle and fat. Little biotransformation occurs; the drug remains in tissues mostly as unchanged apramycin. In general, tissues contained insufficient residues for characterization.

In calf studies, 71% of the radioactivity in kidney and 62% in liver were unchanged apramycin. In pigs, apramycin in kidney ranged from 66–80% of the radioactivity and 85% in the liver samples tested, with exception of two animals where levels of 50% and 36% were found. In chickens, in kidney and liver samples analysed, unchanged apramycin accounted for 80% or more of total activity. It is excreted mainly as unmetabolized apramycin in cattle (>90%) and pigs (75%).

TISSUE RESIDUE DEPLETION STUDIES

Radiolabelled residue depletion studies

Pigs

In radiolabel depletion studies, sampling in the first part was at 4 h and 7 days with six piglets weighing about 8 kg (Herberg *et al.,* 1979), and in the second part, sampling was only done at 14 days, with 6 piglets weighing about 10 kg (Zornes, Herberg and Donoho, 1979). Analysis of fortified ^{14}C apramycin for all control tissues fortified at 0.5, 0.1 and 0.05 mg/kg gave recoveries of all tissues of 98, 107 and 135%, respectively. Balance excretion data at 7 days was 86.9% with 81.6% in faeces and 5.3% in urine. At 14 days the total excreted radioactivity was 82–92% with less than 10% in urine.

Approximately 66% of the radioactivity in kidney was apramycin. None of the other samples contained sufficient residue for characterization. Results are presented in Table 2.1.

Table 2.1. Residues in pig tissues following administration of ^{14}C-apramycin in water (25 mg/kg bw) for 5 days

Withdrawal time	^{14}C Residue (mg apramycin-equivalents/kg)			
	Muscle	Liver	Kidney	Fat
4 hours[(1)]	0.06	0.20	1.39	0.13
	0.22	4.01	70.99	0.30
	0.11	1.94	9.74	0.13
7 days[(1)]	0.04	0.10	0.11	0.09
	0.06	0.19	0.52	0.12
	0.04	0.08	0.17	0.11
14 days[(2)]	0.02	0.08	0.17	0.06
	0.02	0.04	0.05	0.10
	0.05	0.14	0.29	0.15

SOURCES: (1) Herberg *et al.*, 1979. (2) Zornes, Herberg and Donoho, 1979.

Chickens

Zornes, Herberg and Thomson (1985) gave twelve 4-week old Hubbard × White Mountain broiler chickens 500 mg/L ^{14}C-apramycin in the drinking water for five days (maximum recommended dose). Three birds (two male and one female in each group) were terminated at each sampling time of 0 (6 h), 7, 10 and 14 days (body weights of the chickens were not provided). Total radioactivity was determined by direct solubilization followed by liquid scintillation counting. All residue samples were adjusted to 100% counting efficiency. Detection and recovery of radioactivity was checked by fortifying control tissues digested in the same manner as the treated chickens with a nominal radioactivity equivalent to 0.1 mg/kg bw from a solution with a mean value of 109.35 dpm/ml. At zero day withdrawal, average total residues were 3.23, 0.42, 0.20 and 0.07 mg apramycin equivalent/kg in kidney, liver, skin and muscle, respectively. After 14 days, average total residue had declined to 0.47, 0.08, 0.03 and 0.02 mg/kg in the same respective tissues. Apramycin accounted for more than 80% of the total residue in the samples that contained sufficient residue for assay (all kidney samples and the 6 h liver samples). Results are summarized in Table 2.2.

Table 2.2. Residues in chicken tissues following administration of ^{14}C-apramycin in drinking water (500 mg/L) for 5 days

Withdrawal time	^{14}C-Apramycin (mg apramycin equivalents/kg)			
	Muscle	Liver	Kidney	Fat+Skin
0 (6 hours)	0.07	0.24	2.35	0.22
	0.08	0.51	4.47	0.26
	0.07	0.50	2.87	0.13
7 days	0.02	0.20	1.93	0.05
	0.03	0.21	1.60	0.06
	0.02	0.05	0.88	0.06
10 days	0.02	0.14	1.32	0.05
	0.02	0.18	0.70	0.04
	0.02	0.07	0.70	0.04
14 days	0.01	0.11	0.37	0.03

0.02	0.02	0.50	0.04
0.02	0.11	0.55	0.03

Residue depletion studies with unlabelled drug

To assist in the evaluation of the residue depletion studies it is relevant to consider the approved treatments and use of apramycin in pigs, cattle and poultry (Elanco, 2011). The relevant information is provided in Table 2.3 (revised apramycin sponsor submission, 24 October 2011). In some pig studies, the maximum recommended doses in the original dossier did not appear to have been used. The sponsor provided updated information on maximum dose rates in pigs based on regulatory approvals (e.g. Australia and New Zealand) where the highest label dose rate is 25 mg/kg bw/day for neonatal piglets and 12.5 mg/kg bw/day for weanlings. For feed medication in pigs, the maximum dose rate of 8 mg/kg bw/day, using a maximum feed inclusion of 200 mg/kg, implies a voluntary feed intake of 4% of body weight. The sponsor noted that this is a very good estimate for pigs weighing from about 50 kg to slaughter weight. Younger pigs will regularly consume more than 6% of body weight, and as a consequence, higher mg/kg bw/day doses may be found in studies that used younger animals. For example, in the study of Kido *et al*. (1983) noted below, the average weight of the pigs during the period of medication was approximately 26 kg. The pigs consumed approximately 6% of body weight daily, giving average daily doses of 12 and 36 mg/kg bw/day for feed inclusion levels of 200 and 600 mg/kg. As a result, reported studies using neonate or weanling pigs represent considerably higher doses on a mg/kg bw/day basis than would normally be encountered in pigs of market weight.

Table 2.3. Indications and posologies for apramycin

Species	Indication	Formulation	Dose (mg/kg bw/d; inclusion rate in water or feed)	Duration (days)
Calves*	Colibacillosis, Salmonellosis and other bacterial infections	Soluble powder; incorporated in drinking water or milk replacer	20–40	5
		Premix; incorporated in feed	20–40	5
Pigs	Colibacillosis Salmonellosis and other bacterial infections	Soluble powder; incorporated in drinking water	7.5–12.5 (1)	7
		Premix; incorporated in feed	4–8 (80–200 mg/kg feed)	≤ 28
Poultry(2)	*E. coli* septicaemia, Colibacillosis, Salmonellosis and other bacterial infections	Soluble powder; incorporated in drinking water	20–80 (250–500 mg/L)	5–7
		Premix; incorporated in feed	(2–5mg/kg feed)	5
Rabbits	Bacterial enteritis, including colibacillosis	Soluble powder; incorporated in drinking water	10–15 (50–100 mg/L)	5–8
		Premix; incorporated in feed	5–10 (50–100 mg/kg feed)	≤ 21

NOTES: (1) Correction to maximum dose for market-ready pigs. (2) Not for use in animals producing milk or eggs for human consumption.

Additional comment regarding posology of apramycin.

- The highest label dose rate for pigs is 25 mg/kg/day in Australia and New Zealand for neonatal piglets. This dose reverts to 12.5 mg/kg/day for weanlings.
- Most labels require dosing to an inclusion rate in feed or water. Dose rates in terms of mg apramycin per kg per day are typically calculated from "consumption" data, which is often skewed by spillage and waste, particularly in the case of pigs.
- Older animals eat and drink less per body weight than younger animals. Thus, typical market-weight animals will tend toward the lower end of the dose rate range.

It is also relevant to consider the effects of the reported limit of detection (LOD) and limit of quantitation (LOD). The analytical method LOD and LOQ as reported in the sponsor dossier provide preliminary guidance to interpret the results in the residue depletion studies for each animal species and tissue (See Table 2.4). The LOD values were determined in accord with EC directive 93/256EEC, while the LOD values were based on lowest concentration of fortified control tissues used in the method validation and may not reflect true LOQs. Studies conducted in the early 1970s may be reported as analytical range values based on bio-autography and/or paper chromatography. Details regarding the analytical methods are presented in the methods section of this report.

Table 2.4. Limits of detection (µg/kg) and limits of quantitation (µg/kg) of apramycin for different species×matrix combinations (analytical method based on HPLC separation and fluorimetric detection)

Species	Tissue	LOD (µg/kg)	LOQ (µg/kg)	Ratio LOQ/LOD
Cattle	Muscle	268	500	1.9
Cattle	Liver	396	5000	12.6
Cattle	Kidney	229	5000	21.8
Cattle	Fat	129	500	3.9
Pig[1), (2)]	Muscle	280/314	500/500	1.8/1.6
Pig	Liver	250/253	5000/500	20.0/2.0
Pig	Kidney	220/212	5000/2500	22.7/11.8
Pig	Fat	20/23	500/500	25.0/21.7
Pig	Skin+Fat	50/60	500/500	10.0/8.3
Poultry	Muscle	319	500	1.6
Poultry	Liver	470	500	1.1
Poultry	Kidney	133	500	3.8
Poultry	Skin+Fat	32	500	15.6
Rabbit[3), (4)]	Muscle	54/500	500/500	9.2/1.0
Rabbit	Liver	57/100	500/500	8.8/5.0
Rabbit	Kidney	24/600	2500/2500	105/4.2
Rabbit	Fat	38/200	500/500	13.3/2.5

NOTES: (1) Values reported in validation study by Parker, 1995b. (2) Values reported in validation study by Parker, 1995c. (3) Values reported in method validation study by Heal, 2007. (4) Values reported in method validation study by Villa and Brightwell, 1998.

Cattle

Two early studies used a semi-quantitative microbiological method to determine tissue residues after oral administration of apramycin to Holstein bull calves for five days. The calf body weights were 36–52 kg. In each study, three or four calves were terminated at intervals of approximately 1 h, 7, 14, 21 and 28 days after the last dose.

Van Duyn and Handy (1977) treated 16 Holstein dairy calves (body weights 36.3–46.7, mean = 42.1 kg) with bolus doses of 39 mg apramycin/kg bw/day by gavage (maximum recommended dose). Residues in kidney were 50–100 mg/kg at zero day withdrawal, depleting to 1–4 mg/kg after 28 days. Residues in liver were 2–8 mg/kg at zero day withdrawal, depleting to 0.4 mg/kg or less by 21 days. The maximum residue in any muscle sample was 0.5–1.0 mg/kg at zero withdrawal and no residues were detectable by 28 days. Results are summarized in Table 2.5. ND means no residue detected at test sensitivity of <0.1 mg/kg and IS means insufficient sample

Table 2.5. Residues in calf tissues after administration of bolus doses of apramycin (39 mg/kg bw/day) for 5 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	Liver	Kidney	Fat
0 (ca. 1 h)	<0.5	2.0 to 4.0	50.0 to 100.0	IS
	<0.5	4.0 to 8.0	50.0 to 100.0	
	0.5 to 1.0	4.0 to 8.0	50.0 to 100.0	
7 days	<0.1	<0.2	2.5 to 5.0	IS
	0.1 to 0.2	4.0 to 8.0	40.0 to 80.0	
	0.1 to 0.2	4.0 to 8.0	20.0 to 40.0	
14 days	0.1 to 0.2	1 to 2	8.0 to 16.0	IS
	<0.1	2.0 to 4.0	5.0 to 10.0	
	<0.1	2.0 to 4.0	8.0 to 16.0	
21 days	0.1 to 0.2	0.2 to 0.4	10.0 to 20.0	IS
	ND	<0.2	5.0 to 10.0	
	ND	<0.2	5.0 to 10.0	
28 days	ND	<0.2	4.0	IS
	ND	Lost sample	1.0 to 2.0	
	ND	<0.2	4.0	
	ND	<0.2	4.0	

NOTES: ND means no residue detected at test sensitivity of <0.1 mg/kg. and IS means insufficient sample

Table 2.6. Residues in calf tissues after administration of apramycin in milk replacer once daily (40 mg/kg bw/day) for 5 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	Liver	Kidney	Fat
0 (1 hour)	0.1–0.2	2.0–4.0	40.0–80.0	1.0–2.0
	0.1–0.2	2.0–4.0	40.0–80.0	
	0.1–0.2	2.0–4.0	40.0–80.0	
7 days	ND	0.4–0.8	10.0–20.0	0.1–0.2
	<0.05	2.0–4.0	5.0–10.0	
	ND	2.0–4.0	5.0–10.0	
14 days	ND	0.2–0.4	4.0–8.0	IS
	<0.05	1.0	4.0–8.0	
	ND	2.0–4.0	4.0–8.0	
21 days	ND	1.0	1.6–3.2	<0.05
	ND	1.0–2.0	1.6–3.2	
	ND	1.0–2.0	1.6–3.2	
28 days	ND	1.6	2.0–4.0	0.1–0.2
	ND	0.8–1.6	2.0–4.0	
	ND	0.8–1.6	2.0–4.0	

NOTES: ND = no residue detected at the method sensitivity of <0.05– <0.10 mg/kg; IS = insufficient sample.

Handy and Van Duyn (1978) treated 25 × 2–7-day-old calves with apramycin dissolved in reconstituted milk replacer, which was bottle-fed once per day at a dose rate of 40 mg/kg bw (maximum recommended dose). Weights of the calves were 37–52 kg (mean = 42.9 kg) at the beginning of the study. Three animals were sampled at each withdrawal time of 0, 7, 14, 21 and 28 days. Analysis was done using bioautography. Residues in kidney were 40–80 mg/kg at zero withdrawal, depleting to 4 mg/kg or less after 28 days. Residues in liver at zero withdrawal were 2–4 mg/kg, depleting to 1.6 mg/kg or less after 28 days. In muscle, the highest level was 0.2 mg/kg at zero withdrawal and residues were not detected after 21 days. Because of insufficient fat in each animal, a composite fat sample was analysed at each withdrawal time. At zero withdrawal, the residue in fat was 1–2 mg/kg and declined approximately 10-fold by 28 days. The very young age of the animals may not be representative of calves in general because of their immature metabolic status. Results are shown in Table 2.6.

Table 2.7. Residues in male calves following oral dosing with apramycin (40 mg/kg- body weight/day) for 5 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	**Liver**	**Kidney**	**Fat**
0 (4 h)	ND	2.80	118.7	0.900
	ND	0.90	161.8	
	ND	2.00	153.6	
	ND	0.60	75.3	
7 days	ND	ND	12.4	0.100
	ND	ND	15.5	
	ND	1.20	21.7	
	ND	ND	2.80	
14 days	ND	0.40	3.50	ND
	ND	0.40	17.3	
	ND	ND	2.90	
	ND	0.50	3.00	
21 days	0.80	0.60	9.40	ND
	ND	0.60	2.00	
	ND	0.40	3.60	
	ND	0.70	4.40	
28 days	ND	ND	3.90	ND
	ND	ND	1.50	
	ND	ND	1.50	
	ND	ND	0.90	
35 days	ND	0.40	9.20	ND
	ND	ND	2.70	
	ND	ND	0.40	
	ND	ND	1.40	
Tissue LOQ	0.50	5.00	5.00	0.50
Tissue LOD	0.27	0.40	0.23	0.13

NOTES: ND = not detected.

A GLP-compliant residue depletion study in calf tissues was conducted by Parker (1995a). In the two-part study, one group of 20 calves was given 20 mg/kg bw by i.m. injection (Group A) and one group of 24 male Friesian crossbred calves were given oral doses of 40 mg/kg bw apramycin daily (maximum recommended dose for oral treatment) for five consecutive days (Group B). Because oral treatment is the only approved treatment for use, results are only provided for group B (with animals weighing 48–68 kg, mean = 55.5 kg). Groups of four calves were sacrificed at withdrawal intervals of zero (4 h), 7, 14, 21, 28 and 35 days. Apramycin residues in edible tissues were determined using a validated HPLC method (Parker, 1995d). Laboratory analysis provided estimates of residue concentrations that were between the LOQ and the LOD. Residues in liver were always less than the LOQ (5 mg/kg); estimated concentrations were less than 1 mg/kg by 14 days and residues were detected in only one sample collected at 28 and 35 days withdrawal. Because of limited fat, samples at each withdrawal time were composited for analysis. Apramycin was not detected in fat at or beyond the 14 day sample (LOD = 0.13 mg/kg). Residues were not detected in muscle (LOD = 0.27 mg/kg) except one sample at 21 days (<1 mg/kg). Apramycin residues in kidneys were less than 20 mg/kg at 14 days and the majority of the residues were below the limit of quantitation (LOQ = 5.0 mg/kg) by 21 days. Results below the LOQ are estimated values. Results are shown in Table 2.7.

Table 2.8. Residues in calf tissues following oral dosing with apramycin (40 mg/kg bw/day) for 5 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	Liver	Kidney	Fat
7 days	0.35	1.47	4.40	0.33
	0.29	1.71	6.52	0.46
	ND	1.81	6.47	0.14
	0.60	1.48	7.16	0.14
14 days	0.39	1.51	1.57	0.39
	0.52	1.74	1.89	0.84
	ND	1.58	2.21	0.16
	0.43	1.42	3.97	0.62
21 days	ND	2.20	2.97	0.13
	0.27	2.02	2.35	0.23
	0.29	1.77	1.95	0.14
	0.40	1.20	3.37	0.17
28 days	0.35	1.35	1.86	ND
	0.46	1.02	1.79	ND
	0.47	1.48	2.48	ND
	0.58	1.34	2.42	ND
35 days	ND	1.51	2.02	ND
	ND	1.27	2.20	ND
	ND	1.80	3.22	ND
	ND	1.43	1.92	ND
42 days	ND	1.31	1.41	ND
	ND	1.50	1.82	ND
	ND	1.42	2.64	ND
	ND	1.68	3.25	ND
Tissue LOQ	0.50	5.00	5.00	0.50
Tissue LOD	0.27	0.40	0.23	0.13

NOTES: ND = not detected.

An additional GLP-compliant residue study was carried out in young calves (Parker, 1999b), with 24 male and female Friesian crossbred calves given oral doses of 40 mg/kg bw apramycin daily for five days (maximum recommended dose) and groups of four calves were sacrificed at withdrawal intervals of 7, 14, 21, 28, 35 and 42 days. Animal weights were 38–69 kg (mean = 49.0 kg).

Apramycin residues were determined using a validated HPLC method (Parker, 1995d). Apramycin was detected at all withdrawal times in liver and kidney, although residues in liver were all below the LOQ. In kidney, three of the four samples collected at 7 days withdrawal contained apramycin above the LOQ, but no residues above the LOQ were found at 14 days or later withdrawal times. A limited number of muscle and fat samples contained residues above the LOQ at 7 or 14 days or both. Residues were undetectable in fat from 28 days and in muscle from 35 days. Results reported below the validated LOQ are estimated values. Results are shown in Table 2.8.

Residue studies in calves provide some useful data for residue depletion analysis as doses were at the maximum recommended amounts; however, as in the pig studies described below, the time frames are generally too long to provide useful information for residue depletion analysis.

Pigs

In a very old study, 27 pigs were treated with apramycin in drinking water at the recommended dose with 12 serving as controls (and three sacrificed in each sampling timeframe at 4, 7, 14, 28 and 42 days). Pigs were given unlabelled apramycin at 1 g activity per US gallon (approximately 264 mg activity/L) as the sole source of drinking water for 7 days (VPR-164-766, 1972). Body weights of the pigs were not provided, thus dose per kg bw was not reported. Residues in tissues were analysed with a semi-quantitative microbiological assay. No apramycin residues were detected in muscle, skin or fat at any withdrawal interval. The study report shows that the zones of inhibition on the bio-autography plates for these tissues were similar to controls, but the sensitivity of the assay was not defined. There was approximately 0.1 mg/kg in liver at zero withdrawal and <0.1 mg/kg at subsequent time points. Kidney contained ca. 1.3–1.7 mg/kg at zero withdrawal, declining to <0.1 mg/kg by 28 days. Results are tabulated in Table 2.9.

In a similar study, Van Duyn and Johnson (undated) administered apramycin in drinking water at 1 g activity per US gallon (264 mg/l) to 50 piglets for 7 days, with 18 pigs serving as controls and two groups of 16 provided apramycin in the drinking water. The mean initial weight of the piglets was approximately 13 kg. Based on the weight of the piglets and average water consumption, the average daily dose was calculated to be 11.4 mg/lb bw/day, or approximately 25 mg/kg bw/day (200% of maximum recommended dose of 12.5 mg/kg bw/day). Following the seven days of treatment, the medicated pigs were combined into one group of 32 pigs. At days 14, 28, 35 and 42 post-treatment, three randomly selected medicated pigs and two control pigs were sacrificed and tissues collected for residue analysis. No residue above approximately 0.1 mg/kg was found in liver or muscle at any withdrawal interval. One skin+fat sample contained a residue estimated at 0.1–0.2 mg/kg at zero withdrawal (immediately off-treatment), but the other two zero-day samples, and all subsequent withdrawal samples, contained no measurable residue. Kidney contained approximately 1.3 to 5 mg/kg at zero withdrawal, 0.1 to 1 mg/kg at 14 days and <0.1 mg/kg at subsequent withdrawal intervals. Results are in Table 2.10.

Table 2.9. Residues in pig tissues following treatment with apramycin in drinking water (264 mg/l) for 7 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	**Liver**	**Kidney**	**Skin/Fat**
0 days	ND	0.10	1.29	ND
	ND	0.08	1.43	ND
	ND	0.12	1.74	ND
4 days	ND	0.03	1.01	ND
	ND	0.02	0.53	ND
	ND	0.02	0.59	ND
7 days	ND	ND[a]	0.44	ND
	ND	0.03	0.31	ND

	ND	0.06	0.44	ND
14 days	ND	0.01	0.17	ND
	ND	0.04	0.28	ND
	ND	ND	0.16	ND
28 days	ND	ND	ND	ND
	ND	ND	Negligible	ND
	ND	ND	0.05	ND
42 days	ND	ND	ND	ND
	ND	ND	0.03	ND

NOTES: ND = residue not detected (limit of detection not defined)

Table 2.10. Residues in pig tissues following treatment with apramycin in drinking water (264 mg/L) for 7 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	**Liver**	**Kidney**	**Fat**
0 days	ND	ND[a]	2.5–5.0	0.1–0.2
	ND	ND	2.5–5.0	ND
	ND	ND	1.25–2.5	ND
14 days	ND	ND	0.5–1.0	ND
	ND	ND	0.5–1.0	ND
	ND	ND	0.1–0.2	ND
28 days	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	ND	ND
35 days	NA	ND	ND	NA
	NA	ND	ND	NA
	NA	ND	ND	NA
42 days	NA	NA	ND	NA
	NA	NA	ND	NA
	NA	NA	ND	NA

NOTES: ND = residue not detected (limit of detection 0.1 mg/kg); NA = not analysed.

An additional study that used a microbiological assay was conducted in Japan (Kido *et al.,* 1983). Thirty-six castrated male crossbred pigs were medicated with two levels of apramycin in drinking water for 7 days. Actual doses, based on average water consumption and body weights, were approximately 10 and 29 mg/kg bw/day (80% and 230% of the maximum recommended dose). For each treatment level, 3 pigs were slaughtered at 0 (2 h), 7, 14, 21, 28 and 35 days after withdrawal from medication. The sensitivity of the assay was 0.06 mg/kg for all tissues. No residues above the assay sensitivity were detected in muscle or fat from any pig regardless of medication level or withdrawal time. Only one of the liver samples at the 29 mg/kg bw/day treatment level (2 h withdrawal time) contained a residue above the assay sensitivity. Mean kidney residues at 2 h withdrawal time were 0.62 and 1.57 mg/kg for the 10 and 29 mg/kg bw/day dose levels, respectively, while mean levels at 7 days withdrawal were 0.10 and 0.25 mg/kg for the same respective doses. All kidney samples from later withdrawal intervals were below the sensitivity of the assay. Results are provided in Table 2.11.

Table 2.11. Residues in pig tissues following treatment with apramycin in drinking water (10 or 29 mg/kg bw/day) for 7 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	Liver	Kidney	Fat
		Dosed at 10 mg/kg bw/day		
0 (2 h)	ND	ND[a]	0.52	ND
	ND	ND	1.06	ND
	ND	ND	0.27	ND
7 days	ND	ND	0.09	ND
	ND	ND	0.12	ND
	ND	ND	0.08	ND
14 days	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	ND	ND
		Dosed at 29 mg/kg bw/day		
0 (2 h)	ND	ND	1.22	ND
	ND	0.28	1.52	ND
	ND	ND	1.96	ND
7 days	ND	ND	0.20	ND
	ND	ND	0.20	ND
	ND	ND	0.36	ND
14 days	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	ND	ND

A GLP-compliant study was conducted in 1995 using 24 (12 barrow and 12 gilts) Duroc × Landrace piglets medicated with a single daily dose of apramycin in water at 20 mg/kg bw (160% maximum recommended dose, see sponsor comments above on piglets up to 26 kg) by stomach tube for 7 days (Parker, 1995b). Body weights at the beginning of the study were 15–20 kg. Samples of muscle, liver, kidney and skin with fat were collected from four animals at each of the withdrawal periods (1, 4, 7, 14, 21 and 28 days) and residues were determined using a validated HPLC method (Parker, 1995c and addendum). No residues were detected at any withdrawal interval for muscle, liver or fat samples. The validated LOQ for apramycin in kidney was 5 mg/kg, but the authors of the report provided estimates of concentrations that were between the LOD and LOQ. Residues in kidney declined to below the limit of quantitation by 7 days withdrawal. Results are summarized in Table 2.12.

Table 2.12. Residues in pig tissues following administration of apramycin in water (20 mg/kg bw) for 7 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	Liver	Kidney	Skin+fat
1 day	ND	ND	5.80	ND
	ND	ND	6.90	ND
	ND	ND	15.30	ND
	ND	ND	6.30	ND
4 days	ND	ND	4.50	ND

	ND	ND	3.60	ND
	ND	ND	5.80	ND
	ND	ND	6.40	ND
7 days	ND	ND	2.60	ND
	ND	ND	2.90	ND
	ND	ND	3.10	ND
	ND	ND	2.50	ND
14 days	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	0.70 (1)	ND
	ND	ND	ND	ND
21 and 28 days	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	ND	ND
Tissue LOQ	0.50	5.00	5.00	0.50
Tissue LOD	0.28	0.25	0.20	0.05

Notes: ND = not detected. (1) Estimated value.

The results of these studies show that oral treatment of pigs with apramycin results in very low concentrations of residues in edible tissues. The older studies that used microbiological assays are in general agreement with newer studies that used HPLC methods of analysis. Zero-day withdrawal kidney residues were lower after natural intake of drinking water containing apramycin, compared with single daily bolus doses given by gavage.

In an early study, 27 pigs were fed a ration medicated with 100 g apramycin activity per U.S. ton (110 mg/kg) for 56 days (Experiment SW-396, 1971) (55% of maximum recommended treatment in feed). Two males and one female were terminated at 0, 4, 7, 14, 28, 37 and 42 days after withdrawal of the medicated ration; six pigs served as controls. Body weights of the pigs were not reported. Analysis of tissues using a semi-quantitative microbiological assay found no detectable residue of apramycin in muscle, fat or skin at any withdrawal time. Residues in kidney were estimated to be approximately 2–3.4 mg/kg at zero withdrawal, depleting to negligible or undetectable levels by 28 days. Residues in liver were always less than 0.1 mg/kg; they were detected in all samples through 7 days withdrawal, in none of the pigs at 14 days, in one pig at 28 days and in no animals at 37 or 42 days. Results are provided in Table 2.13.

Table 2.13. Residues in pig tissues following treatment with apramycin in feed (110 mg activity/kg) for 56 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	**Liver**	**Kidney**	**Fat**
0 day	ND	0.07	3.40	ND
	ND	0.09	3.40	ND
	ND	0.08	2.00	ND
4 days	ND	0.04	0.50	ND
	ND	0.04	0.85	ND
	ND	0.05	0.38	ND
7 days	ND	0.03	0.19	ND
	ND	0.03	0.30	ND
	ND	0.04	0.27	ND

14 days	ND	ND	0.09	ND
	ND	ND	0.03	ND
	ND	ND	0.25	ND
28 days	ND	ND	ND	ND
	ND	Negligible	ND	ND
	ND	ND	ND	ND
37 days	ND	ND	ND	ND
	ND	ND	Negligible	ND
	ND	ND	ND	ND
42 days	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	ND	ND

Notes: (1) Limit of detection in liver not defined.

In another study (Handy and Van Duyn, 1979), pigs of mixed breed and sex, ranging in weight from 8 to 22 kg, were fed a ration medicated with 110 mg/kg apramycin activity for 28 days (55% of maximum recommended treatment in feed). Six randomly selected pigs were slaughtered at 0 (approximately 1 h) 7, and 14 days after withdrawal of the medicated ration and three randomly selected pigs were terminated at 21, 28 and 35 days after withdrawal. For the 0, 14 and 28 day intervals, tissue samples from two pigs were composited before analysis. Apramycin residues were determined using a semi-quantitative assay involving thin layer chromatography and bio-autographic detection with *Bacillus subtilis*. No residues were found above the test sensitivity of 0.1 mg/kg in fat+skin or muscle tissues at any withdrawal time. Kidneys contained 0.5 to 1.0 mg apramycin/kg at zero withdrawal, depleting to 0.2 mg/kg at 7 and 14 days; no residue was detected in kidney after 21, 28 or 35 days withdrawal. Liver contained <0.1 mg/kg at zero withdrawal; except for a trace of residue in one of the 3 samples analysed after 7 day withdrawal, no other liver sample contained a detectable apramycin residue. The report states that, based on the average feed consumption during 28 days and the final weight of the pigs, the average dose of apramycin was 2.83 mg/kg bw/day (23% of the maximum recommended dose/kg bw/day). However, if the calculation is based on the average weight of the pigs during medication, the mean dose is approximately 4.65 mg/kg bw/day. Results are presented in Table 2.14.

Table 2.14. Residues in pig tissues following treatment with apramycin in feed (110 mg activity/kg feed) for 28 days

Withdrawal time	**Apramycin (mg/kg) LOD = 0.1 mg/kg**			
	Muscle	**Liver**	**Kidney**	**Fat**
0 (1 hour)	ND	<0.1	0.5–1.0	ND
	ND	<0.1	0.5–1.0	ND
	ND	<0.1	0.5–1.0	ND
7 days	ND	ND	0.1	ND
	ND	<0.05	0.1–0.2	ND
	ND	ND	0.1–0.2	ND
14 days	ND	ND	0.1–0.2	ND
	ND	ND	0.1 -0.2	ND
	ND	ND	0.1 -0.2	ND
21, 28 and 35 days	NA	ND	ND	NA
	NA	ND	ND	NA
	NA	ND	ND	NA

NOTES: ND = not detected; NA = not analysed.

The study of Kido *et al.* (1983), discussed earlier, also included treatment groups given apramycin in feed. Thirty-six castrated male crossbred pigs were fed a ration medicated with 200 or 600 mg/kg apramycin *ad libitum* (100% or 300% of the maximum recommended treatment in feed). Actual doses, based on average feed consumption and average body weights, were 12 and 36 mg/kg bw/day (150 and 450% of the maximum recommended dose). Three pigs were slaughtered at 0 (2 h), 7, 14, 21, 28 and 35 days after withdrawal from treatment. The sensitivity of the assay was 0.06 mg/kg for all tissues. No residue was detected in muscle or fat from any pig regardless of dose level or withdrawal time. In kidney, residues at 2 h withdrawal were 0.38–0.54 mg/kg for the 12 mg/kg bw/day dose and from 0.97–1.63 mg/kg for the 36 mg/kg bw/day dose. No residues were detected in kidney at 7 days or later withdrawal intervals. No residues were detected in liver from pigs that received the 12 mg/kg bw/day dose at any withdrawal time. Liver samples from two of the pigs that received 36 mg/kg bw/day and terminated at 2 h withdrawal contained 0.18 or 0.07 mg/kg apramycin. Liver from the third pig terminated at 2 h and from all pigs at later withdrawal intervals contained no residue above the sensitivity of the assay. Results are provided in Table 2.15.

A GLP marker residue study was conducted in pigs using apramycin premix at the highest recommended dose (Parker, 1999a). Sixteen Large White Cross pigs, 12 weeks old (plus one control pig) with body weights 19–31 kg (mean = 21.9 kg) at the initiation of the study were dosed at a nominal rate of 200 mg apramycin/kg of feed for 28 days (body weights of 28–52 kg [mean = 34.7 kg] at the end of the study). Estimated daily doses based on feed consumption ranged from 13.1 to 16.7 mg/kg bw (105–134% of the maximum recommended dose/kg bw/day). Samples of muscle, liver, kidney and skin with fat were collected from four animals at withdrawal periods of 3, 6, 9 and 12 days. Residues were determined using the validated HPLC method that reported the LOQ in liver tissue at 0.5 mg/kg, lower than in other studies. In this study, in contrast to other pig studies, the highest residues were found in liver tissue. The results are summarized in Table 2.16.

Table 2.15. Residues in pig tissues following treatment with apramycin in feed (12 or 36 mg/kg bw/day) for 7 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	Liver	Kidney	Fat
		12 mg/kg bw/day dosage		
0 (2 h)	ND	ND	0.43	ND
	ND	ND	0.54	ND
	ND	ND	0.38	ND
7 and 14 days	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	ND	ND
		36 mg/kg bw/day dosage		
0 (2 h)	ND	0.18	0.97	ND
	ND	ND	1.11	ND
	ND	0.07	0.63	ND
7 and 14 days	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	ND	ND

NOTES: ND = not detected.

Table 2.16. Residues of apramycin in pig tissues following administration of apramycin in feed (200 mg/kg feed) for 28 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	Liver	Kidney	Skin/Fat
3 days	ND	1.2 8	0.39	ND
	ND	1.31	0.83	ND
	ND	1.25	ND	ND
	ND	1.40	ND	ND
6 days	ND	1.55	ND	ND
	ND	1.41	ND	0.16
	ND	1.62	ND	0.17
	ND	1.43	1.31	0.14
9 days	ND	1.37	0.41	ND
	ND	1.20	1.39	0.16
	ND	1.22	0.79	0.14
	ND	1.09	ND	0.13
12 days	ND	1.14	ND	0.19
	ND	0.99	ND	0.18
	ND	1.02	ND	0.33
	ND	1.22	0.53	0.19
Tissue LOQ	0.50	0.50	2.50	0.50
Tissue LOD	0.31	0.25	0.21	0.06

NOTES: ND = not detected

The apramycin residue studies of pigs following treatment in feed are in general agreement with the pattern of distribution observed in studies with apramycin through drinking water or by gavage dosing, with residues monitored through ^{14}C-labelling, HPLC or microbiological assay. Residues are usually highest in kidney, followed by liver (see, however, Table 2.16) with residues depleting rapidly after withdrawal of treatment. Residues are generally not detectable or very low in muscle and skin+fat. Each of the studies, however, is deficient in the multiple day selection of withdrawal times, compromising assessment of residue depletion studies.

Chickens

Thirty-six Hubbard crossbred broiler chickens (12 males and 12 females in trial; 12 birds as controls), approximately 30 days old, were administered apramycin in drinking water (559 mg/L, 110% maximum recommended treatment, in drinking water) for five days (Handy and Thomson, 1985). Three males and three females were terminated at 0 (5 h), 7, 10, 14, 21 and 28 days after withdrawal of medication, and residues in edible tissues were determined using a microbiological assay. Based on use of medicated water and the total pen bird weights on the day before treatment was initiated, the average dose was estimated to be 102 mg/kg bw/day (127% of maximum recommended dose). Weights of the individual groups of chickens were 1.36 kg at day 0; 1.75 kg at day 7; 2.02 kg at day 10; 2.12 kg at day 14; 2.71 kg at day 21; and 2.93 kg at day 28. Five hours after withdrawal, residues of apramycin in kidney were 2.7–6.9 mg/kg; these declined to ≤1 mg/kg after 7 days, and to ≤0.48 mg/kg after 28 days. Residues in liver were 0.26–0.54 mg/kg after 5 h withdrawal, ≤ 0.21 mg/kg after 7 days and <0.05 mg/kg by 21 and 28 days. Residues in skin were 0.06–0.2 kg/kg after 5 h and no residues were detected at later withdrawal intervals. In this study the authors used a method labelled as AM-AA-CA-R100-AA-775. The reported LOQ was 0.05 mg/kg in all tissues with the exception of 7 and 10 day withdrawal skin samples with a LOQ of 0.1 kg/kg. These LOQs are notably lower than other apramycin methods and studies. One fat sample at 5 h withdrawal contained 0.15 mg/kg; the remaining samples, and all samples from subsequent samplings, contained no detectable residue. Residues in muscle were <LOQ at all withdrawal intervals. An earlier study using a less developed microbiological assay gave similar results (Handy, 1985). Results are presented in Table 2.17.

Because of limited sample sizes, tissues from chickens were sometimes pooled before analysis. Skin and fat were analysed separately and the value shown in the table is the higher of the two values. Below LOQ means <0.05 mg/kg, except 7-day skin tissue samples where the LOQ = 0.01 mg/kg. No residue detected (ND) means no response below the LOQ (0.01 mg/kg).

In a GLP-compliant study, apramycin was administered in drinking water at 500 mg/L to 48 four-week-old Ross broiler chickens for 5 days (Parker, 1998a). Body weights were tabulated for each of the four sampling points (day 3 birds, 0.98 kg; day 6 birds, 0.95 kg; day 9 birds, 1.03 kg; and day 12 birds, 0.92 kg). Delivery of medicated water was also calculated: day 1, 118.7 mg/kg bw; day 2, 94.4 kg/kg bw; day 3, 112.0 mg/kg bw; day 4, 132.1 mg/kg bw; day 5, 134.3 mg/kg bw). The average dose was estimated to be 118 mg/kg bw/day (about 150% of the maximum recommended dose). Tissues from ten birds were analysed for apramycin using a validated HPLC method (Parker, 1998b). The reported LOQ was 5 mg/kg for all tissues (see Table 2.4). Allowing for the differences in withdrawal times and the precision of the assays, the results of the chicken radiometric residue studies and the microbiological method studies are in general agreement with the Parker (1998a) study using the HPLC assay. Results are reported in Table 2.18.

Table 2.17. Residues in chicken tissues following administration of apramycin in drinking water (559 mg/L) for 5 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	**Liver**	**Kidney**	**Skin/Fat**
0 (5 h)	BLQ	0.26	3.30	0.20
	ND	0.35	6.90	0.11
	ND	0.33	—	0.08
	BLQ	0.54	8.40	0.06
	ND	0.29	2.70	0.09
	ND	0.35	—	0.10
7 days	ND	0.21	1.00	ND
	ND	0.14	0.56	ND
	ND	0.08	—	ND
	ND	0.09	0.79	ND
	ND	0.06	0.97	ND
	ND	0.11	—	ND
10 days	ND	0.15	0.81	ND
	ND	0.08	0.59	ND
	ND	0.06	—	ND
	ND	BLQ	1.05	ND
	ND	0.11	0.53	ND
	ND	0.12	—	ND
14 days	NA	0.08	0.45	NA
	NA	0.12	0.58	NA
	NA	0.16	—	NA
	NA	BLQ	0.82	NA
	NA	0.05	0.61	NA
	NA	0.23	—	NA
21 days	NA	BLQ	0.49	NA
	NA	BLQ	0.88	NA
	NA	BLQ	—	NA
	NA	BLQ	0.52	NA
	NA	BLQ	0.37	NA
	NA	BLQ	—	NA
28 days	NA	BLQ	0.14	NA
	NA	ND	0.31	NA
	NA	BLQ	—	NA
	NA	BLQ	0.31	NA
	NA	BLQ	0.48	NA
	NA	ND	—	NA

NOTES: NA = not analysed; BLQ = below limit of quantitation; ND = not detected.

Table 2.18. Residue in chicken tissues following administration of apramycin in drinking water (500 mg/L) for 5 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	Liver	Kidney	Skin+fat
3 days	ND	ND	BLQ	BLQ
	ND	ND	BLQ	0.62
	ND	ND	0.85	BLQ
	ND	ND	0.58	BLQ
	ND	ND	1.48	BLQ
	ND	ND	0.62	BLQ
	ND	ND	0.90	BLQ
	ND	BLQ	1.10	0.55
	ND	ND	BLQ	BLQ
	ND	ND	0.66	ND
6 days	ND	ND	1.03	ND
	ND	ND	BLQ	ND
	ND	ND	1.40	ND
	ND	ND	0.77	BLQ
	ND	ND	0.62	ND
	ND	ND	0.54	BLQ
	ND	ND	BLQ	BLQ
	ND	ND	BLQ	BLQ
	ND	ND	BLQ	ND
	ND	ND	BLQ	BLQ
9 days	ND	ND	BLQ	ND
	ND	ND	0.56	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	0.60	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	ND	ND
	ND	ND	BLQ	ND
12 days	ND	ND	BLQ	ND
	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	ND	ND
	ND	ND	ND	BLQ
	ND	ND	BLQ	ND
	ND	ND	0.58	ND
	ND	ND	BLQ	ND

Notes: ND = not detected; BLQ = below limit of quantitation.

Rabbits

In a GLP-compliant study, apramycin was administered in the drinking water at 100 mg/L (maximum recommended dose) for 7 days to 30 New Zealand White rabbits. Weight ranges at the beginning of the study for males was 2.2–2.5 kg and for females, 2.2–2.6 kg. The estimated mean concentration of medicated water was determined to be 108.6 mg/L/day for the 7-day study. Liver, kidney, muscle and fat were analysed for apramycin using a validated HPLC method with a LOQ of 0.50 mg/kg (Villa and Brightwell, 1998). One liver sample at day zero withdrawal had a value of 0.6 mg/kg; all other samples at day zero were <LOD. All day 3 and subsequent withdrawal time samples were <LOD. Results are in Table 2.19

Table 2.19. Residues in rabbit tissues following administration of apramycin in drinking water (100 mg/L) for 7 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	Liver	Kidney	Fat
0	ND	0.600	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	ND	ND
3, 7, 14 and 21 days	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	ND	ND
Tissue LOQ	0.50	0.50	2.50	0.50
Tissue LOD	0.50	0.10	0.60	0.20

NOTES: ND = not detected; BLQ = below limit of quantitation.

In a second GLP compliant study, 36 New Zealand White rabbits were medicated with apramycin at nominal rates of 100 mg/kg bw or 300 mg/kg bw in the feed (100% or 300% of the maximum recommended treatment dose) for 21 days (Heal, 2008). Calculated medicated doses were 85.6 mg/kg and 258 mg/kg in feed. Body weights of the rabbits at the beginning for the study were 0.9–1.5 kg (mean 1.36 kg). The achieved apramycin dose was 6.6 ± 1.0 mg/kg bw/day for the low-dose group and 20.1 ± 2.3 mg/kg bw/day for the high-dose group. At the end of the 21-day study, mean body weight of the low-dose group was 2.07 kg and the high-dose group was 2.16 kg. Feed intakes were calculated for each group at day 21 of treatment: for the low-dose group the estimated daily feed intake was 138.1 g/day and the high-dose group was 139.7 g/day. The overall mean medicated daily intake of apramycin in the low-dose group was 11.82 mg/kg bw/day and for the high-dose group the mean was 37.0 mg/kg bw/day. Muscle, liver, kidney and fat were sampled from six animals at each of the withdrawal periods (0, 24 and 48 h). In both treatment groups, all samples were below the LOD for muscle (49.5 µg/kg), liver (51.7 µg/kg) and fat (34.3 µg/kg). In the high-dose group, all but three kidney samples contained residues between the LOD (21.7 µg/kg) and the LOQ (2.28 mg/kg), while the remaining three kidney samples residues were non-detectable. All kidney samples in the low-dose group had non-detectable residues. Residues were determined using a validated HPLC fluorescence method (Heal, 2007). Because of the limited number of positive residue findings, only those from the high treatment group are reported in Table 2.20.

These studies indicate that apramycin is very poorly absorbed by rabbits, either by medication in drinking water or in medicated feed.

Table 2.20. Residues in rabbit tissues following administration of apramycin in feed (300 mg/kg feed) for 7 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	Liver	Kidney	Fat
0 hours	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
24 hours	ND	ND	ND	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
48 hours	ND	ND	BLQ	ND
	ND	ND	ND	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	ND	ND
Tissue LOQ	0.50	0.50	2.50	0.50
Tissue LOD	0.05	0.06	0.02	0.04

NOTES: ND = not detected; BLQ = below limit of quantitation.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

During the last decade, several laboratories have worked on the development of analytical methods for the analysis of aminoglycosides (e.g. Cheng *et al.,* 2010; Ishii *et al.,* 2008; Bogialli *et al.,* 2005; van Holthoona *et al.,* 2009; Stead, 2000). These methods cover a wide range of matrices (mainly for which MRLs have been established). Generally, most protocols use an extraction solvent containing trichloroacetic acid and sample clean-up is performed with solid phase extraction (SPE) on weak cation exchange cartridges such as CBA or CBX. Chromatographic separation is commonly performed using reversed-phase ion-pair principles. Although some groups describe the use of ultraviolet (UV) detection and fluorescence detection (FD), the lack of a suitable chromophore requires a derivatization step to detect aminoglycosides. Chemiluminescence has been described as a rapid and robust detection technique for aminoglycosides without the need for derivatization. Mass spectrometric detection has the same benefits as chemiluminescence but with higher selectivity and sensitivity especially in the selective reaction mode (SRM) of a triple quadrupole LC–MS system. Most methods use tobramycin as an internal standard, because deuterated or chemical analogues of the aminoglycosides are not available. Some research groups have synthesized internal standards (such as dimethylspectinomycin, methyldihydrostreptomycin and octamethylkanamycin A) that provide incomparable performance for quantification.

Methods used in absorption and bio-availability studies

Regarding methods submitted by the sponsor, blood levels (in relevant species) were investigated by a microbiological assay. This assay used Mueller-Hinton (Difco) media adjusted to pH 8.0 and *Bacillus*

subtilis ATCC 6633 as test organism, against apramycin residues in tissue. The paper disc procedure was used. Plates were kept overnight at ambient temperature and were then incubated at 37°C until distinct growth of the assay organism was apparent. Semi-logarithmic plots of known apramycin concentrations versus diameters of inhibition zones were linear with typical correlation coefficients (r) >0.93. The intra-day and inter-day variability were <7.5% and <2.5% through the concentration ranges studied. Few additional technical details are given in the experimental section of the studies. The semi-quantitative microbiological assay is adapted to produce qualitative data, i.e. 'presence or absence'. No quantitative measurement is available by this approach [see Van Duyn and Johnson (undated) "…the assay for aprymicin … does not provide for the establishment of a finite residue level for each sample… The assay is more applicable to verifying the absence of apramcyin than the exact measurement of residue level…"]. The threshold of detection was established at 0.1 mg/kg (standard recovery samples fortified at 1.0 mg/kg could always be distinguished from negative control tissue samples), but details (e.g. number of replicates and concentrations studied) are lacking. The semi-quantitation is done by measuring the spot intensity onto the plate. The nature of the bio-autographic tissue residue procedure is more appropriately suited to the detection of antimicrobial residues rather than the estimation of amount present. The semi-quantitative residue activity estimations are frequently reported as activity ranges. It is difficult to know if the apramycin residues were found to be highly variable between animals (the dose response was difficult to assess) due to individual animal variability or to the analytical method itself. The specificity of the detection is not described; false negative and false positive rates are unknown.

Methods used in residue depletion studies

The data used in the residue depletion (distribution) studies were provided mainly by a LC-fluorescence detection method. Tissue is treated with ammonium hydroxide/methanol solution to release apramycin. The methanolic solution is evaporated to dryness, re-suspended in aqueous buffer, and ion-pair extracted into ethyl acetate/di-(2-ethylhexyl)phosphate (DEHP). Apramycin is back extracted into 0.75M aqueous hydrochloric acid and subsequently neutralized with sodium hydroxide. The neutralized aqueous solution is then washed with toluene and aliquots transferred to vials for analysis. Apramycin is determined by HPLC with fluorescence detection after pre-column derivatization with o-phthaldehyde. The reaction acts on the apramycin primary amines to produce a fluorescent imine derivative (see Figure 2.1).

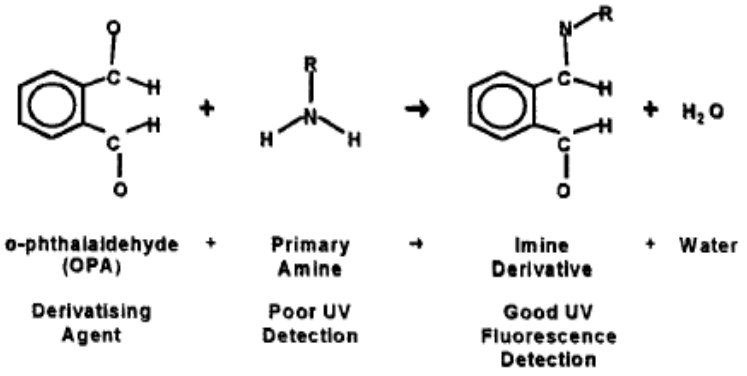


Figure 2.1. The reaction with the apramycin primary amine to produce a fluorescent imine derivative

All compounds bearing an amino group will be modified by the derivatization step, and will produce a detectable signal by the fluorescence detector. Any residual molecule from the clean-up procedure containing a similar primary amine will also undergo the derivatization step. No internal standard is used in the method.

The specificity is passable to medium, as attested by the chromatograms shown in Figure 2.2 (copied from the sponsor submission) for different blank and fortified kidney extracts (0.5 mg/kg and 1.0 mg/kg of apramycin).

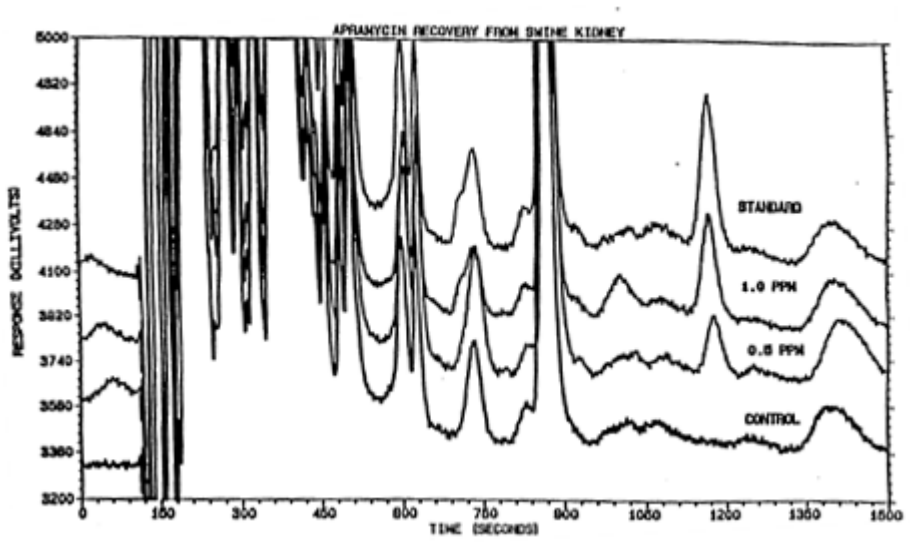


Figure 2.2. Chromatograms corresponding to pig kidney extracts (blank, 0.5 mg/kg and 1.0 mg/kg fortified samples, standard)

Limits of detection have been calculated as described in European Community Decision 93/256 (replaced in 2002 by the 2002/657/EC decision), i.e. on the calculation of the noise observed in blank tissues plus 3 standard deviations of the noise. Twenty chromatograms of negative control tissue were used to determine the LODs. They are generally good (see Table 2.4), especially for fat or skin+fat samples. LODs in liver and muscle are less satisfactory; sometimes the offset (20 mU in the region of elution of apramycin) is high for liver extracts (a complex matrix and the cleanliness of the extract being worse).

The LOQ for the sponsor-based methods used the lowest concentration for which the method has been validated to a stated level of confidence (first point of calibration). For this reason, LOQs are sometimes very high and are probably far from the true analytical LOQs. Reasonably, and without additional information provided by the studies, the Committee considered it possible to deduce (or estimate) LOQs from LODs by applying a basis of blank tissue mean residue finding plus ten standard deviations, resulting in a factor of approximately LOQ/LOD = 3. The Committee also critically reviewed the quality of the analytical tracings of the residue validation studies in its evaluation of method performance. Accordingly, the Committee re-calculated the LODs and LOQs in the different tissues for the different species for the studies provided by the sponsor. The analytical signal information was taken on representative chromatograms available in the submission. The method of calculation was performed according to the principle shown in Figures 2.3 and 2.4.

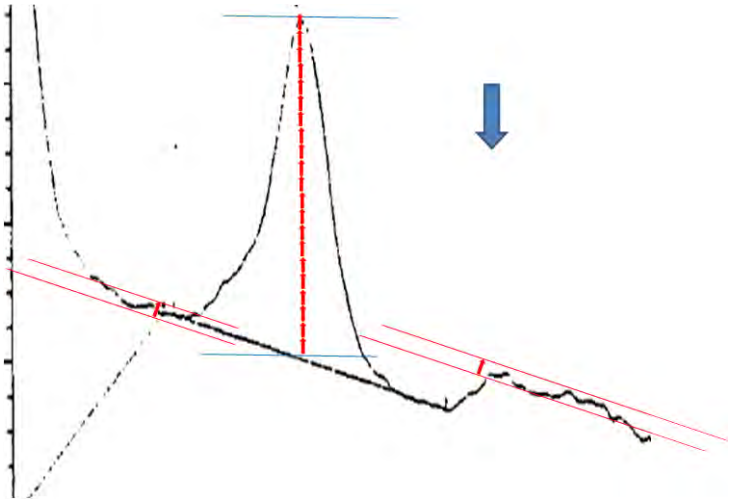


Figure 2.3. Method applied to re-assess LODs and LOQs. Illustration given on a chromatogram corresponding to a blank porcine skin+fat fortified with apramycin at 1 mg/kg (Parker, 1995c).

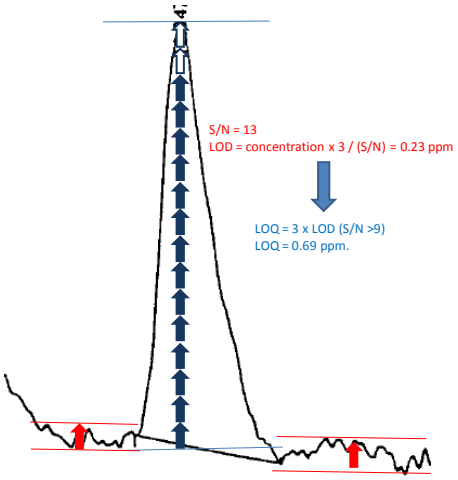


Figure 2.4. Method applied to re-assess LODs and LOQs. Illustration given on a chromatogram corresponding to a blank bovine fat fortified with apramycin at 1 mg/kg.

The noise has been measured in a retention time region as close as possible to the target signal of apramycin (i.e. retention time ± 5 peak widths at 50% height of the peak). The determination of the noise amplitude was determined to be as representative as possible, avoiding any over- or under-estimation of the corresponding value. The LOD has been calculated at a signal-to-noise ratio of 3. The LOQ has been deduced by applying a factor of 3 to the LOD. A summary of the values are given in Table 2.21 below. Assessment of the signal has been given in two separate columns; the offset level gives an indication of the probable complexity of the extract whereas the signal quality refers to the interpretability of the chromatographic peak (resolution, co-elution, etc). Finally, depending on the quality of the chromatograms (the consequence of both the PDF version readability and method specificity), a confidence level has been attributed to the interpretation; it reflects the level of certainty we may have on the LOD/LOQ recalculation. LOD/LOQ values are given in mg/kg.

Table 2.21. Summary of the committee-calculated values of LODs and LOQs in representative blank tissues fortified in different species' chromatograms from the sponsor submission.

SPECIES	TISSUES	LOQ SPONSOR	LOQ REVISITED	LOD SPONSOR	LOD REVISITED	OFFSET LEVEL	SIGNAL QUALITY	CONFIDENCE IN ASSESSMENT	STUDY REFERENCES
Cattle	Liver	5000	5000	396	1700	LOW	GOOD	GOOD	Parker 1995d
Cattle	Kidney	5000	2600	229	900	LOW	GOOD	GOOD	Parker 1995d
Cattle	Muscle	500	750	268	250	VERY HIGH	WEAK	GOOD	Parker 1995d
Cattle	Fat	500	370	129	230	VERY HIGH	GOOD	GOOD	Parker 1995d
Pig	Liver	5000***/500***	2500***/900***	253***/250***	830***/300***	MEDIUM***/LOW***	GOOD***/GOOD***	GOOD***/GOOD***	Parker 1995c/***Parker 1995c Addendum***
Pig	Kidney	2500	1900	212	625	MEDIUM	MEDIUM	MEDIUM	Parker 1995c
Pig	Muscle	500	1800	314	600	HIGH	BAD-COELUTION	LOW	Parker 1995c
Pig	Skin/fat	500	450	60	150	HIGH	MEDIUM	MEDIUM	Parker 1995c
Pig	Fat	500	390	23	130	MEDIUM	GOOD	GOOD	Parker 1995c
Poultry	Liver	500	750	133	250	MEDIUM	BAD-COELUTION	MEDIUM	Parker 1998b
Poultry	Kidney	500	1300	470	430	MEDIUM	BAD-COELUTION	MEDIUM	Parker 1998b
Poultry	Muscle	500	1140	319	380	MEDIUM	GOOD	MEDIUM	Parker 1998b
Poultry	Skin/fat	500	480	32	160	LOW	GOOD	GOOD	Parker 1998b
Rabbit	Liver	500***/500***	1500***/4500***	57***/100***	500***/1500***	LOW***/MEDIUM***	MEDIUM***/WEAK***	GOOD***/GOOD***	Heal(2008)/***Villa and Brightwell (1998)***
Rabbit	Kidney	2500***/2500***	3750***/9000***	24***/600***	1250***/3000***	LOW***/LOW***	MEDIUM***/GOOD***	LOW***/GOOD***	Heal(2008)/***Villa and Brightwell (1998)***
Rabbit	Muscle	500***/500***	*900* ***/9000***	54***/500***	*300* ***/3000***	LOW***/LOW***	MEDIUM***/GOOD***	MEDIUM***/WEAK***	Heal(2008)/***Villa and Brightwell (1998)***
Rabbit	Fat	500***/500***	120***/1800***	38***/200***	40***/600***	LOW***/LOW***	EXCELLENT***/GOOD***	GOOD***/WEAK***	Heal(2008)/***Villa and Brightwell (1998)***

Reproducibility studies were not routinely carried out in the validation exercise. Repeatability (recovery) has been assessed through intra- and inter-batch variations. The recovery has been calculated at different concentration levels and in different batches of samples [64% in liver (Repeatability standard deviation (rsd) = 10%), 70% in kidney (rsd = 7%), 68% in muscle (rsd = 11%), 77% in fat (rsd = 9%) and 74% in skin+fat (rsd = 10%)]. The repeatability is generally acceptable.

Most recent methods are based on extraction by a solvent containing trichloroacetic acid; this method unexpectedly used ammonium hydroxide and methanol to release target residues from tissue. Instead of using an SPE ion exchange strategy, the method used a complex liquid-liquid extraction with an ion-pair agent. In conclusion, the analytical methods combine a non-specific purification followed by a weak specific detection (i.e. all co-extracted 'amino interfering compounds'), derivatized generating a fluorescent signal resulting in a more complex chromatogram, and generating results that are more difficult to interpret. The only identification criterion is the chromatographic retention time. Internal standards are not used for identification and quantification of apramycin.

The quality of the data generated by the LC-fluorescence detector method is of medium quality, but may be used for the risk assessment exercise. The Committee is more reserved regarding use of the data generated from the microbiological assay.

A limited set of quality criteria (Table 2.22) were applied to each sample batch (retention time, repeatability of the standard signal, linearity of the calibration curve, recovery).

Table 2.22. Acceptance criteria used to validate batches of samples

Acceptance criterion	Action if unacceptable
1. Retention time of analyte peak within 20 seconds of nearest reference standard	Sample negative.
2. Percentage coefficient of variation of standards through run >10	Re-run HPLC. If still unacceptable, run batch again with fresh standards.
3. Regression of matrix curve <0.950	Re-extract batch.
4. Percentage recovery within the acceptable range for that particular species+tissue type. (see validation reports)	Re-extract batch.

APPRAISAL

Apramycin is an old drug with a long history of use. It has not been reviewed previously by the Committee. Apramycin is a broad spectrum aminocyclitol antibiotic produced stereospecifically by a strain of *Streptomyces tenebrarius*. It is extracted from the fermentation medium as apramycin sulphate at a purity of at least 85% and total impurities are not to exceed 15%. Six impurities have been identified and one impurity, 3 O-hydroxyapramycin ($C_{21}H_{41}N_5O_{12}$), has a biological spectrum that is very similar to apramycin, with microbiological activity of one-half to one-quarter compared with apramycin. A microbiological assay was used to determine activity as equivalents of apramycin base.

Apramycin is used in veterinary medicine, effective against both Gram-positive and Gram-negative bacteria, some strains of mycoplasma and most field strains of *E. coli* and *Salmonella* spp.. It is bactericidal at minimum inhibitory concentrations. The drug exerts its antibacterial effect by inhibiting protein synthesis at the level of peptidyl translocation. It is mostly used for treating gastrointestinal infections. Apramycin is available in soluble powder and feed premix formulations.

In calves, apramycin is intended to be administered at a dose of 20 to 40 mg/kg bw/day in drinking water, milk replacer or feed for 5 days. In pigs, it is intended to be administered at a dose of 7.5 to 12.5 mg/kg bw/day in drinking water or as premix incorporated in feed at a dose of 4 to 8 mg/kg bw/day (80–200mg/kg feed) for less than 28 days. In poultry, it is intended to be administered at a dose of 20 to 80 mg/kg bw/day (250 to 500 mg/L) in drinking water for 5–7 days or as premix incorporated in feed at a dose of 2 to 5 mg/kg feed for 5 days. In rabbits, it is intended to be administered at a dose of 10 to 15 mg/kg bw/day (50 to 100 mg/L) in drinking water for 5 to 8 days or as premix incorporated in feed at a dose of 5 to 10 mg/kg bw/day (50–100 mg/kg feed) for less than 21 days. It is not to be used in animals producing eggs or milk for human consumption.

Apramycin is a weak organic base which is highly polar with low solubility in lipids and a poor ability to penetrate membranes. Metabolism data are consistent across species. Levels in blood after oral dosing were much lower than after parenteral treatment, indicating low oral availability in pigs, calves and chickens. In calves, pigs, chicken, rabbits, rats and dogs, apramycin is rapidly and poorly absorbed by the oral route and quickly eliminated. Main levels in serum are found a few hours after treatment (until 6 h) and are undetectable between 24 and 36 h. Pharmacokinetic studies indicate oral availability of approximately 3% in pigs and 2% in chickens. Oral doses are extensively excreted in faeces (more than 82% in pigs and 99.5% in rats) while parenteral doses are mostly excreted in urine in a low percentage of the given dose (11% for cattle, less than 10% for pigs, rats and dogs). Binding of apramycin to serum proteins was 26%.

Chromatographic analysis of the studies with radiolabelled apramycin did not identify any major metabolites in blood, excreta or tissues. Most of the radioactivity in blood, urine, faeces and tissues was unmetabolized apramycin. The distribution of residues in edible tissues was similar for oral and

parenteral routes of administration, but levels were much lower with oral treatment. Kidney contained the highest concentration of residue, followed by liver. Muscle and fat (or skin+fat) contained little or no apramycin residue. Kidney was generally the tissue from which apramycin depleted most slowly. In cattle, pigs and chicken, levels of radioactivity are highest in kidney, followed by liver and very minor quantities in muscle and fat. Little biotransformation occurs, the drug remains in tissues mostly as unchanged apramycin. In general, tissues contained insufficient residues for characterization.

Sixteen residue depletion studies (both GLP-compliant and studies prior to GLP regulations) were provided by the sponsor: four in young calves, eight in young pigs, two in chickens and two in rabbits. However, as there were no regulatory approvals noted by the sponsor for rabbits, these are not included in the appraisal (i.e. 14 studies are considered). A summary of residue study findings at or above the reported LOQ is tabulated in Table 2.23, irrespective of the time points (see individual studies for details).

Table 2.23. Summary of residue findings above reported limits of quantitation in four9teen residue depletion studies

Animal	Study report	Muscle	Liver	Kidney	Fat or Skin+fat
Calves	Van Duyn and Handy, 1977	Values reported as ranges	Values reported as ranges	Values reported as ranges	0
	Handy and Van Duyn, 1978	Values reported as ranges	Values reported as ranges	Values reported as ranges	Values reported as ranges
	Parker, 1995a	1	0	10	1
	Parker 1995b	3	0	3	2
Pigs	VPR-164-766, 1972	0	0	0	0
	Van Duyn and Johnson, undated	0	0	0	0
	Kido *et al.,* 1983 (drinking water)	0	0	6 at 2.3× recom-mended dose	0
	Parker 1995b	0	0	6 at 1.6× recom-mended dose	0
	SW-396, 1971	0	0	0	0
	Handy and Van Duyn, 1979	0	0	0	0
	Kido *et al.,* 1983 (feed)	0	0	0	0
	Parker, 1999a	0	16	0	0
Chicken	Handy and Thompson, 1985(1)	0	22	24	6
	Parker, 1998a	0	0	0	0

NOTES: (1) Reported LOQs were 0.05 mg/kg for all tissues except 7 and 10 day skin+fat

In these fourteen studies, with the exception of one study, the majority of the positive residue values were in kidney tissues. Liver tissue contains the second highest concentrations of residues in tissue, and in one pig study liver contained the highest amounts of apramycin. Residues in muscle and fat or fat+skin were universally very low. These conclusions are consistent with the radiolabel studies. The studies indicate that apramycin is the suitable marker residue and kidney as the appropriate target tissue.

Of primary concern with the analytical methods is the disparity in how the limit of detection and limit of quantitation were determined. Limits of detection were based on the calculation of the noise observed in blank tissues plus 3 standard deviations of the noise. Twenty chromatograms of negative control tissue were used to determine the LODs. They are generally good expected values, especially for fat or skin+fat samples. LODs in liver and muscle are less good; sometimes the offset (20 mU in

the region of elution of apramycin) is high for liver extracts (a complex matrix) and the cleanliness of the extract is poor. The LOQ approach employed by the sponsor studies used the lowest concentration to which the method has been validated to a stated level of confidence, and consequently may not represent true limits of quantitation. This can be readily seen in Table 2.4. The Committee therefore re-calculated method performance LOD and LOQ values and re-assessed the positive residue findings in the studies. In reviewing the residue findings for estimating recommendations on MRLs, the Committee concluded that it was limited to applying the previously described statistical procedures from the 66th and 70th meetings of the Committee. Only two tissue residue data sets were available that provided sufficient positive residue findings greater than the reported LOQ. They were calf kidney and chicken kidney. The results are presented in the Figures 2.5 and 2.6, respectively.

The consequence of the concerns relating to the analytical methods and the limited set of residue determinations for the fourteen studies in calves, pigs and chickens present a complex determination of recommended MRLs for apramycin.

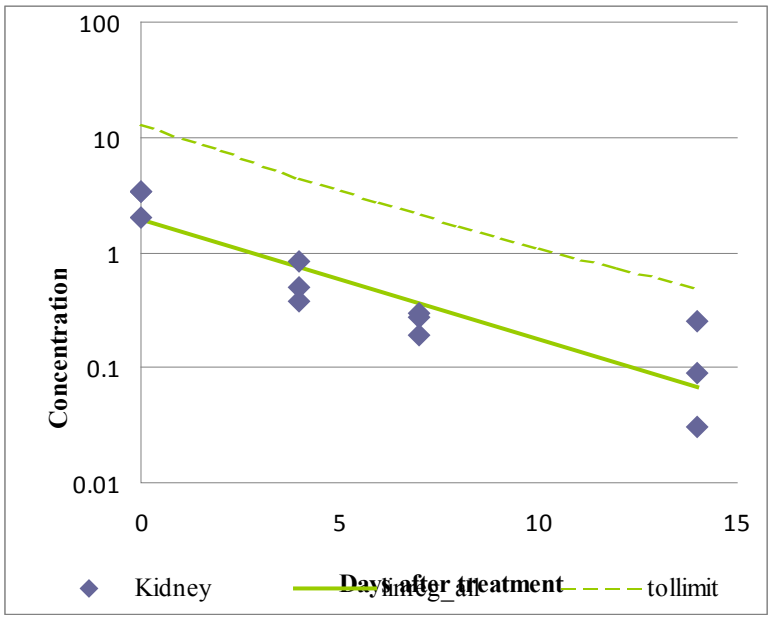


Figure 2.5. Tolerance limit considerations for calf kidney residues of apramycin

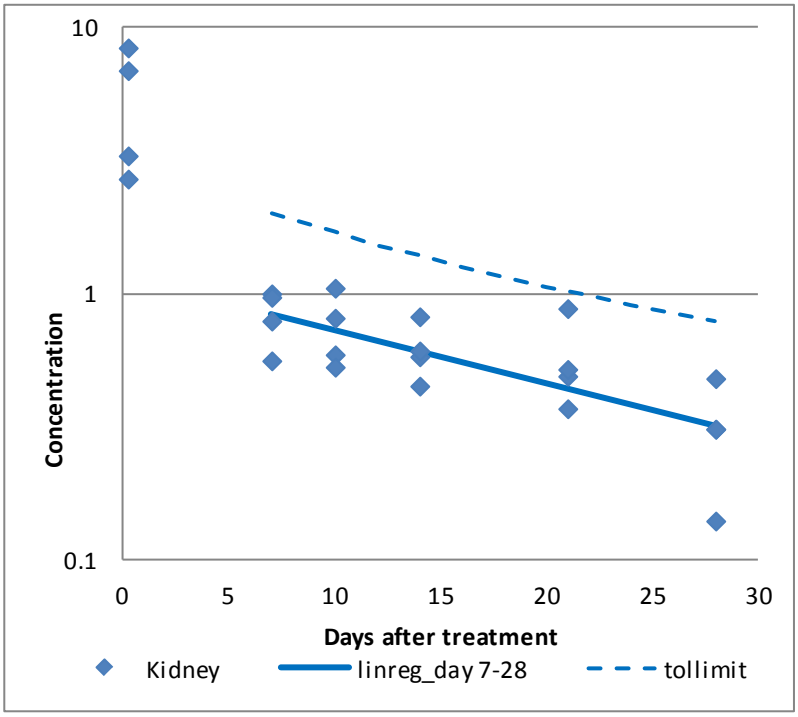


Figure 2.6. Tolerance limit considerations for chicken kidney residues of apramycin

MAXIMUM RESIDUE LIMITS

In recommending MRLs for apramycin, the Committee considered the following factors:

- A microbiological based acceptable daily intake (ADI) was established at 0–30 μg/kg bw, equivalent to an upper bound of 1800 μg per day for a 60 kg person.
- Apramycin is produced as a fermentation product and acceptable purity is ≥85%.
- Apramycin is poorly absorbed orally in calves, pigs and chickens.
- LOQs revised by the Committee were used to identify the values in the residue depletion studies which could be used for the assessment.
- Considering the revised LOQs, in four calf studies, 3 muscle, 24 kidney and 5 fat values were greater than the LOQ; in eight pig studies, 16 liver and 15 kidney samples were greater than the LOQ with almost all at doses of 1.6–2.3 times the recommended dose; in chicken, 24 kidney samples were above the LOQ.
- There are only sufficient residues above the LOQs in calf kidney and chicken kidney to estimate tolerance limits based on statistical approaches. In all other species and tissues, the low number of reported values above the respective LOQs made unachievable the assessment of tolerance limits based on statistical approaches.
- Residues are consistently highest in kidney tissues in the residue depletion studies, with the exception of one study. Kidney is the appropriate target tissue.
- Apramycin remains mostly unchanged and is therefore the appropriate marker residue.

The Committee recommended temporary MRLs at 5 mg/kg only in cattle and chicken kidney, measured as apramycin based on statistical approaches. If the MRLs were calculated according to the LOQs provided by the sponsor or the LOQs re-calculated by the Committee, the maximum estimated daily intake of apramycin residues in the worst case scenario would be around 1400 μg/day and would not exceed the upper bound of the ADI.

The sponsor is requested to provide improved analytical methods with better performance with lower LOQs, and residue depletion studies with appropriate sampling points close to the zero withdrawal periods for all tissues and species. The validated analytical method(s) and residue depletion studies are requested by the end of 2014.

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Derquantel

First draft prepared by
Lynn G. Friedlander, Rockville, MD, USA
Bruno Le Bizec, Nantes, France
and
Gerald E. Swan, Pretoria, Republic of South Africa

IDENTITY

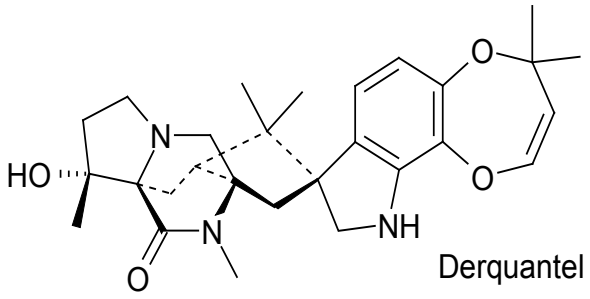
International Non-proprietary names (INN): Derquantel

Synonyms: PF-00520904, PNU-141962, 2-DOPH, 2-desoxyparaherquamide, 2-deoxyparaherquamide, Startect® (derquantel + abamectin)

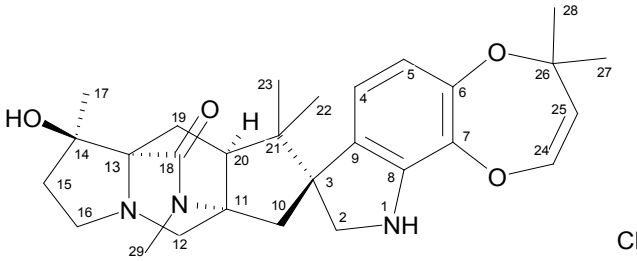
IUPAC Name: (1*R*,3*S*,5*R*,7*S*,12*R*)-12-Hydroxy-4,4,4',4',12,14-hexamethyl-9',10'-dihydro-4'*H*spiro[9,14-diazatetracyclo [5.5.2.$0^{1,9}$.$0^{3,7}$]tetradecane-5,8'-[1,4]dioxepino[2,3-*g*]indol]-13-one

Chemical Abstract Service Number: 187865-22-1

Structural formula of main components:



Derquantel



Chiral

Molecular formula: $C_{28}H_{37}N_3O_4$

Molecular weight: 479.6

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Appearance: White to tan coloured powder, essentially free from foreign matter

Melting point: 196.5–197.5 °C

Solubility:

From Gottschall, 2010a	From MSDS, 2009
125 mg/L in water at 25°C	0.1–1 mg/L in water
	0.1–1 mg/ml in n-heptane
21.6 mg/ml in isopropanol at 25°C	10–33 mg/ml in propanol
29.1 mg/ml in tert-amyl alcohol at 25°C	33–100 mg/ml
10.0 mg/ml in tert-butylmethylether at 25°C	1-10 mg/ml
55.9 mg/ml in ethyl acetate at 25°C	
54.8 mg/ml in methanol at 25°C	100–1000 mg/ml
39.6 mg/ml in acetonitrile at 25°C	
0.1 mg/ml in isooctane at 25°C	
79.4 mg/ml in toluene at 25°C	
	33–100 mg/ml in acetone

RESIDUES IN FOOD AND THEIR EVALUATION

Conditions of use

Derquantel, a spiroindole, is an oral anthelmintic for use in sheep. It is registered only in combination with abamectin. The combination product contains 10 mg/ml derquantel and 1 mg/ml abamectin. Derquantel, in combination with abamectin, is used to treat and control a broad range of adult and immature (L4) gastrointestinal nematodes of sheep, including parasites resistant to macrocyclic lactone-, levamisole-, benzimidazole- and closantel-based drenches (and combinations of these) (Kaminsky *et al.,* 2011; Little *et al.*, 2010, 2011).

Dosage

The dose for sheep and lambs is 2 mg derquantel and 0.2 mg abamectin/kg bw.

PHARMACOKINETICS AND METABOLISM

Pharmacokinetics in laboratory animals and humans

No *in vivo* pharmacokinetic studies are provided for derquantel in laboratory animals or humans. A report from the published literature (Aloysius *et al.,* 2008) describes the comparative pharmacokinetics of paraherquamide, a closely related compound, in dogs, gerbils and sheep (Tables 3.1 and 3.2). The GLP status of this study cannot be determined from the literature report.

Table 3.1. Pharmacokinetics of paraherquamide and total drug related material (DRM) in sheep and dogs following a single 0.5 mg/kg oral dose of ^{3}H-paraherquamide

Pharmacokinetic parameter	Sheep		Dog	
	PHQ	Total DRM	PHQ	Total DRM
C_{max} (ng/ml)	38	46	73	167
T_{max} (h)	0.5	8	0.5	1
$AUC_{0\text{-}last}$ (ng-h/ml)	547	1192	136	1387
$t_{1/2}$ (h)	8.5	13	1.5	23

Table 3.2. Excretion of radioactivity in sheep, dogs and gerbils following a single oral dose of ^{3}H-paraherquamide

	Sheep	Dog	Gerbil
Dose (mg/kg)	0.5	0.5	20
Collection time (h)	0–96	0–72	0–72
Urine	9%	23%	17%
Faeces	81%	58%	67%
Total	90%	81%	84%

Pharmacokinetics in food-producing animals

Sheep

Two GLP-compliant pharmacokinetic studies were conducted in sheep, the target species for the commercial combination product (derquantel plus abamectin).

A GLP-compliant pharmacokinetic study (Walker, 2009b) was conducted in Texel and Texel-cross sheep to compare pharmacokinetic parameters after oral or intravenous dosing. Twelve sheep were assigned (3 castrated males, 3 intact males and 6 females) per dose group. Group 1 received the final commercial formulation (2 mg derquantel/kg bw and 0.2 mg abamectin/kg bw) by drench. Group 2 received an i.v. injection of derquantel alone at a dose of 1.0 mg/kg bw, equivalent to half the dose of derquantel in the commercial combination product formulation. Group 3 received an i.v. injection of abamectin at a dose level of 0.1 mg/kg bw, again equivalent to half the dose of abamectin in the commercial combination product formulation. Plasma samples were collected at pre-dose and for 168 hours post-dose. A validated LC-MS/MS method, with an LOQ of 0.5 ng/ml for each analyte, was used to quantify derquantel and abamectin in plasma. The pharmacokinetic parameter estimates for derquantel and abamectin are presented in Tables 3.3 and 3.4, respectively.

Table 3.3. Pharmacokinetic parameters of derquantel in plasma of sheep (males+females) following i.v. (derquantel alone) and drench (commercial combination product) administration

Parameter	Oral commercial combination product			i.v. Derquantel		
	Mean	95% Confidence Interval		Mean	95% Confidence Interval	
		Lower	Upper		Lower	Upper
C_{max} (ng/ml)	108	80.8	145	959	716	1280
T_{max} (hours)	4.17	3.25	5.10	0.182	-0.741	1.11
$t_{1/2}$ (hours)	9.3	6.1	19.3	5.7	4.0	9.7
AUC $_{0\text{-last}}$ (ng-h/ml)	1760	1330	2340	1570	1390	1780
AUC $_{0\text{-}\infty}$ (ng-h/ml)	1790	1360	2360	1590	1410	1810
VD_{SS} (ml/kg)	NC	NC	NC	3220	2470	3970
Clearance (ml/kg/min)	NC	NC	NC	11.1	9.49	12.8
Bio-availability (%)	56.3	44.3	71.4	NC	NC	NC

NOTES: NC = not calculated.

Table 3.4. Pharmacokinetic parameters of abamectin in plasma of sheep (males+females) following oral administration of abamectin alone or in the commercial combination formulation

Parameter	Oral commercial combination product		i.v. Abamectin	
	Mean	95% Confidence Interval	Mean	95% Confidence Interval

		Lower	Upper		Lower	Upper
C_{max} (ng/ml)	31.1	22.5	42.9	109	79.0	150
T_{max} (hours)	23.8	17.8	29.8	0.122	0.029	0.216
$t_{1/2}$ (hours)	27.7	18.0	59.9	27.6	18.0	59.6
AUC $_{0\text{-last}}$ (ng-h/ml)	1660	1220	2260	1170	865	1590
AUC $_{0\text{-}\infty}$ (ng-h/ml)	1730	1250	2390	1240	898	1710

There was no statistically significant (P>0.05) sex-related differences in any pharmacokinetic estimates between castrated males, intact males and females for derquantel or abamectin, and all data represent combined male+female results. The derquantel in the combination product had a T_{max} of 4.17 h (range 3.25–5.10 h) and a C_{max} of 108 ng/ml (range 80.8-145 ng/ml). The oral bio-availability of derquantel in the combination product was approximately 56% (range 44.3-71.3%). Following i.v. administration, derquantel was well distributed in the body with a volume of distribution at steady state (VD_{SS}) of 3220 ml/kg (range 2470-3970 ml/kg) and a moderate clearance of 11.1 ml/kg/min (9.5-12.8 ml/kg/min). Derquantel, in the combination product, had a half-life of 9.3 h (range 6.1–19.3 h). The maximum plasma concentration of abamectin was reached in about 24 h after a single oral dose of the combination product. The oral bio-availability was about 70%. Abamectin was well distributed in the body with a VD_{SS} of 3530 ml/kg. Clearance of abamectin was low, at 1.5 ml/kg/min. The half-life was 27.6 h.

In a second GLP-compliant plasma pharmacokinetic study (Walker, 2009a), the potential for interaction between derquantel and abamectin, when co-administered by oral drench as the commercial combination product, was evaluated in Texel and Texel-cross sheep. Three treatment groups were evaluated: derquantel alone, abamectin alone, and the commercial combination product formulation containing 2 mg/kg bw derquantel and 0.2 mg/kg bw abamectin. Each treatment group contained 6 males and 6 females. Plasma samples were collected at pre-dose and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, 24, 36, 48, 72, 96, 120, 144 and 168 h after dosing. Samples were analysed using a validated LCMS/MS method. The method has an LOQ of 0.5 ng/ml for each analyte in plasma.

With the exception of T_{max} derquantel, there were no statistically significant (P>0.05) sex differences in any pharmacokinetic parameters for either derquantel or abamectin. The T_{max} for derquantel for males was 3.96 (2.84–5.07) h and for females was 2.65 (1.53–3.76) h. Although there was a statistically significant difference in derquantel plasma T_{max} between males and females, T_{max} was considered similar and did not result in a sex difference in overall exposure when defined by the $AUC_{0\text{-t(last)}}$, $AUC_{0\text{-}\infty}$, and C_{max}.

All other comparisons were made using combined male and female data. There were no statistically significant differences (P>0.05) for any pharmacokinetic parameter between dose groups for either derquantel or abamectin with the exception of $AUC_{0\text{-t(last)}}$ for derquantel (P=0.044). The T_{max} (combined) was 2.60 h (range 1.05–4.16 h) for derquantel in the combination product versus 4.00 for derquantel alone. For the commercial combination, the half-life for derquantel was 13.1 h (range 9.82–19.6 h) versus 10.4 h for derquantel alone. The $AUC_{0-\infty}$ was 1250 ng-h/ml (range 1000–1570 ng-h/ml) for the combination product versus 1710 ng-h/ml for derquantel alone. The C_{max} for derquantel in the combination product was 92.8 ng/ml (range 68.2–126 ng/ml) in the second study versus 105 ng/ml for the derquantel alone.

The half-life for abamectin in the combination product (27.7 h in the first study and 25.4 h in the second study) is consistent with that found during the evaluation of abamectin at the 45th Meeting of the Committee (1.2 ± 0.3 days) (FAO, 1995; 1996) and is generally consistent with the somewhat more variable half-life results reported in the scientific literature (Irish Medicines Board, 2007; Hongbo *et al.,* 1998). See Table 3.4A.

Table 3.4A. Half-life values for abamectin reported in the literature

Parameter	Source of data		
	Walker, 2009b.	**Hongbo *et al.*, 1998.**	**Irish Medicines Board, 2007**
Dose (mg/kg)	0.2	0.2	0.2
C_{max} (ng/ml)	31.1	21.2	44
T_{max} (h)	24	14	24
$t_{1/2}$ (h)	28 (17.9-56.5)*	69.9	61.8 (12-192)

NOTES: * = mean (range)

Table 3.5. Pharmacokinetic parameters of derquantel in plasma of sheep (males+females) following oral administration of derquantel alone or in the commercial combination product formulation

Parameter	Oral commercial combination product			Oral Derquantel		
	Mean	95% Confidence Interval		Mean	95% Confidence Interval	
		Lower	Upper		Lower	Upper
C_{max} (ng/ml)	92.8	68.2	126	105	76.9	143
T_{max} (hours)	2.60	1.05	4.16	4.00	2.45	5.55
*t*1/2 (hours)	13.1	9.82	19.6	10.4	9.25	12.0
AUC $_{0\text{-last}}$ (ng-h/ml)	1210	974	1500	1680	1310	2140
AUC $_{0\text{-}\infty}$ (ng-h/ml)	1250	1000	1570	1710	1340	2180

Table 3.6. Pharmacokinetic parameters of abamectin in plasma of sheep (males+females) following oral administration of abamectin alone or in the commercial combination product formulation

Parameter	Oral commercial combination product			Oral Abamectin		
	Mean	95% Confidence Interval		Mean	95% Confidence Interval	
		Lower	Upper		Lower	Upper
C_{max} (ng/ml)	26.5	21.9	32.1	26.1	21.5	31.6
T_{max} (hours)	23.5	17.1	29.9	16.8	7.8	25.9
*t*1/2 (hours)	25.4	19.1	38.1	22.1	17.2	31.2
AUC $_{0\text{-last}}$ (ng-h/ml)	1670	1170	2400	1190	831	1700
AUC $_{0\text{-}\infty}$ (ng-h/ml)	1730	1190	2520	1240	849	1800

Taken together, the overall evidence indicates no significant interaction between derquantel and abamectin when dosed in combination. Pharmacokinetic parameters for derquantel are summarized in Table 3.5 (see also EMA, 2010). Pharmacokinetic parameters for abamectin are summarized in Table 3.6.

Two disposition studies using radioisotopically labelled derquantel (one non-GLP-compliant pilot study and one GLP-compliant study) in sheep were evaluated.

In the non-GLP pilot disposition study (Byrd and Liu, 2006), sheep were treated orally by capsule with ^{14}C-derquantel to achieve a target dose of 2 mg derquantel/kg bw. In Phase 1 of the study (one female), 6 capsules were needed to achieve the target dose due to the low specific activity of the test article. In Phase 2 (four males, one per timepoint), material with a higher specific activity was used and only one capsule per sheep was needed. The Phase 1 animal was slaughtered 3 h after dosing. The Phase 2 animals were slaughtered 6, 12, 24 and 48 h after dosing.

Faeces, urine and cage rinse samples were collected beginning on day 1 (both phases) and continuing until slaughter. Blood was collected immediately prior to slaughter. Whole blood was combusted and analysed by liquid scintillation counting (LSC). Plasma, urine and cage rinse samples were analysed directly by LSC. Faeces were homogenized, combusted and analysed by LSC.

The majority of the radioactivity was eliminated in the faeces at each sacrifice interval after 12 h. In the first 24 h, approximately 50% of the radioactivity was recovered in the excreta: 42% in the faeces and 8% in the urine. By 48 h, approximately 85% of the radioactivity was recovered in the excreta: 55% in the faeces and 30% in the urine (Table 3.7).

Table 3.7. ^{14}C-derquantel equivalent residues (µg/kg) from sheep treated with a single oral dose of ^{14}C-derquantel at a dose of 2 mg/kg bw

Group	Slaughter interval after dosing	Urine	Faeces	Bile	Whole blood	Plasma	Cage rinse
1	3 h	—	20	NA	147.1	178.9	1.8
2	6 h	5 405	875.1	—	337.7	349.1	665.9
3	12 h	13 125	11 968	34 572	222.2	220.3	1 015
4	24 h	6 160	47 975	26 682	58.3	49.1	925.6
5	48 h	2 128	28 584	8 323	17.9	14.0	427.6

NOTES: NA = not analysed.

In the GLP-compliant disposition study (Byrd and Liu, 2008), 20 crossbred sheep (n=3 per group) were treated orally by gastric tube with ^{14}C-derquantel. The specific activity of the test article was adjusted so that animals in the later slaughter groups received derquantel with the higher specific activity. All animals received a single oral dose of approximately 2.10 mg derquantel/kg bw. Animals were slaughtered approximately 6, 12, 24, 48, 96 and 144 h after dosing. Samples were stored at -20°C until analysed.

Urine, faeces, plasma, blood, bile and cage rinses were collected from each sheep daily, beginning on day (-1) and continuing through slaughter. Urine, plasma, bile and cage rinses were analysed directly by LSC. Whole-blood samples were analysed directly by combustion and LSC. Faeces were homogenized and submitted for combustion and LSC analysis.

The average total recovery of the administered dose in Groups 1, 2, 3, 4, 5 and 6 were 7.0%, 24.1%, 49.2%, 88.3%, 89.5% and 91.8%, respectively. Most of the administered radioactivity was recovered in the faeces, followed by urine and liver (for full tissue disposition, see Figure 3.1 and Table 3.8). The total radiolabelled residue (TRR) in urine (plus cage rinse) and faeces accounted for approximately 10% and 85% of the dose administered, respectively (see Figure 3.1 and Table 3.8).

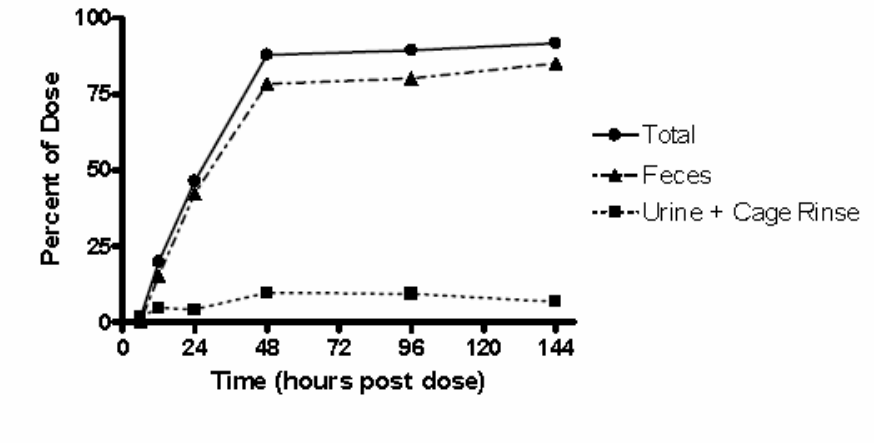


Figure 3.1. Cumulative recovery of ^{14}C-Derquantel equivalents (μg/kg)

No disposition data are provided for abamectin following use of the combination product. Although plasma and biliary data for abamectin were not included with the earlier Committee evaluation (FAO, 1995), it was concluded that, with 90% dose recovery, the majority (70%) was recovered in faeces. Both Committee evaluations (FAO, 1995, 1996) noted that abamectin, at the time it was reviewed, was registered only for use in beef cattle.

Table 3.8. Average total ^{14}C-derquantel equivalents*(μg/kg) in urine, faeces, bile, whole blood, plasma and cage rinse in sheep given a single oral dose of 2.10 mg ^{14}C-derquantel/kg bw

Group	Slaughter interval post-dose	Urine	Faeces	Bile	Whole blood	Plasma	Cage rinse
1	6 h	827.4	490.7	NA	176	217	474.2
2	12 h	3 161	28 339	34 572	106	119	348.3
3	24 h	1 996	45 128	26 682	84	89	705.3
4	48 h	2 701	43 513	8 323	11	10	840
5	96 h	20.7	1 606	56	5	2	16
6	144 h	6.7	169	14	6	2	4.7

NOTES: * = Average is for the specific slaughter time only

Metabolism in rats, sheep, dogs and humans

Two GLP-compliant studies were conducted to evaluate the metabolism of derquantel in the rat, sheep, humans and dogs.

In a GLP-compliant metabolism study (Ma, 2006a), radiolabelled desoxoparaherquamide (derquantel) was incubated with rat, sheep, dog and human liver microsomes. Non-radiolabelled 7-ethoxycoumarin (7-EC) was included as a positive control to test the metabolic integrity of the enzymatic preparations. After a 1-hour incubation, 55.0, 67.6, 100.0, and 75.0% of 7-EC was metabolized by rat, sheep, dog and human liver microsomes, respectively. A significant amount of 7-hydroxycoumarin (7-HC), a metabolite of 7-EC, was observed in 1-hour incubation samples and, consequently, all microsomal preparations were determined to be enzymatically active.

Incubation mixtures of ^{14}C-2-desoxoparaherquamide were extracted with methanol and analysed for metabolites using radio-HPLC. HPLC eluants were collected at 0.25 minute intervals using a fraction collector and were analysed for radioactivity using LSC. Radioactivity peaks were integrated to determine the percent distribution of individual metabolites in each sample.

The data show that ^{14}C-2-desoxoparaherquamide (derquantel) was extensively metabolized by dog liver microsomes and moderately metabolized by rat, sheep and human liver microsomes (Tables 3.9 and 3.10). After a 1-hour incubation, approximately 45.2, 39.8, 2.8 and 55.0% of the radioactivity was

attributable to unchanged drug in rat, sheep, dog and human liver microsomes, respectively. In addition to unchanged ^{14}C-2-desoxoparaherquamide, 18 radioactive components were detected in rat, sheep, dog or human liver microsomal incubations. The predominant metabolites after 1-hour incubation included M1, M9, M10 and M12 and accounted for about 9.5, 3.6, 7.3 and 3.4% of the radioactivity in the rat; 12.9, 5.3, 6.1 and 4.0% in sheep; 2.6, 2.1, 5.5 and 2.9% in the dog; and 12.4, 7.1, 8.9, and 3.6% in humans, respectively. M2, which accounted for approximately 2.1, 3.9 and 1.7% of the radioactivity in 1-hour rat, sheep and human liver microsomal incubations, respectively, was not detected in 1-hour dog liver microsomal incubations. Similarly, M3, a predominant metabolite accounting for approximately 40.1% of radioactivity in 1-hour dog liver microsomal incubations, accounted for only 2.4 and 7.6% of radioactivity in 1-hour rat and sheep liver microsomal incubations. Recovery of radioactivity was high at all sampling times, 0.25–1.0 h, and in all species: greater than 98% in the rat; nearly 95% in sheep; greater than 95% in dogs; and greater than 97% in humans.

Table 3.9. Relative percentage distribution of radioactivity (%HPLC)[(1)] in methanol extracts from rat, sheep, human and dog liver microsomes incubated for 0.25 hours at 37°C with ^{14}C-derquantel[(2)]

Metabolite	Species			
R*t* (min)	Rat	Sheep	Human	Dog
2.6–3.6	ND	1.40	ND	2.28
11.4–12.4	ND	ND	ND	2.05
13.4–14.5	0.73	2.28	ND	20.85
14.0–15.6	ND	1.08	ND	6.54
15.4–17.1	ND	ND	ND	4.52
17.9–19.6	ND	ND	ND	7.16
19.9–21.6	ND	ND	ND	1.44
21.9–24.6	0.94	ND	ND	ND
24.1–26.4	0.86	ND	ND	ND
26.9–28.4	8.37	1.94	ND	10.22
27.9–29.9	1.99	1.24	3.71	1.93
29.4–31.9	3.59	2.87	3.96	3.32
31.9–33.1	ND	ND	ND	ND
36.1–38.1	3.05	2.95	3.21	3.28
39.4–41.1	1.62	1.08	1.92	1.14
41.1–43.1	69.59	73.52	79.99	30.13
45.1–47.1	7.06	7.67	5.17	3.73
52.5–54.6	1.10	0.99	0.88	ND
62.9–64.9	ND	ND	ND	ND
Total (%)	98.90	97.02	98.84	98.59

Notes: (1) Average of triplicate samples. (2) ^{14}C-derquantel at 1 μM. ND = not detectable.

In a similarly designed GLP-compliant study (Ma, 2006b), ^{14}C-2-desoxoparaherquamide (derquantel) was incubated with cryopreserved hepatocytes from rats, dogs and humans, and freshly prepared hepatocytes from sheep. Non-radiolabelled 7-ethoxycoumarin (7-EC) and 7-hydroxycoumarin (7-HC) were included as positive controls to test the metabolic integrity of the hepatocyte preparations. Derquantel incubated in the absence of hepatocytes served as a negative control. The viability of the control hepatocytes was checked after 0, 2 and 4 h of incubations. At the completion of the 4-hour incubation, 36.4% of the rat hepatocytes and 70.0% of the sheep, dog and human hepatocytes remained viable.

The stability of ^{14}C-derquantel (1 μM and 25 μM concentrations) was assessed in incubations with media for 0 h and 4 h. At the 1 μM concentration, only 69.5% and 62.9% of unchanged parent

remained in the 0 and 4 h incubations, respectively. At the 25 μM concentration, 90.4% and 90.1% of unchanged parent remained in the 0 and 4 h incubations, respectively.

The positive controls were used to determine the enzymatic activity of the hepatocyte preparations. After a 4-hr incubation, 35.4, 94.6, 45.8, and 29.5% of 7-EC and 18.5, 100, 96.3, and 90.4% of 7-HC were metabolized by rat, sheep, dog, and human hepatocytes, respectively. Sulphate and glucuronide conjugates also were identified. The hepatocyte preparations were deemed enzymatically active.

Table 3.10. Relative percentage distribution of radioactivity (%HPLC)[(1)] in methanol extracts from rat, sheep, human and dog liver microsomes incubated for 1 hour at 37°C with ^{14}C-derquantel[(2)]

Metabolite	**Species**			
R*t* (min)	**Rat**	**Sheep**	**Human**	**Dog**
2.6–3.6	0.98	4.43	0.98	5.55
11.4–12.4	ND	ND	ND	2.99
13.4–14.5	2.39	7.62	ND	40.09
14.0–15.6	ND	2.10	ND	8.34
15.4–17.1	ND	1.02	ND	8.68
17.9–19.6	ND	ND	ND	7.58
19.9–21.6	ND	ND	ND	2.22
21.9–24.1	2.47	ND	ND	ND
24.1–26.4	1.44	1.44	ND	ND
26.9–28.4	13.96	2.67	ND	4.21
27.9–29.9	3.57	5.25	7.05	2.14
29.4–31.9	7.33	6.06	8.89	5.47
31.9–33.1	1.85	ND	1.35	ND
36.1–38.1	3.44	3.96	3.58	2.94
39.4–41.1	3.18	2.47	4.87	ND
41.1–43.1	45.23	39.82	55.04	2.78
45.1–47.1	9.46	12.86	12.43	2.60
52.5–54.6	1.27	1.31	1.54	ND
62.9–64.9	2.09	3.88	1.69	ND
Total (%)	98.66	94.89	97.42	95.59

NOTES: (1) Average of triplicate samples. (2) ^{14}C-derquantel at 1 μM. ND = not detectable.

Incubation mixtures of ^{14}C-2-desoxoparaherquamide were extracted with methanol and analysed for metabolites using radio-HPLC. HPLC eluants were collected at 0.25 minute intervals using a fraction collector and were analysed for radioactivity using LSC. Radioactivity peaks were integrated to determine the percent distribution of individual metabolites in each sample.

Table 3.11. Relative percentage distribution of radioactivity (%HPLC)[(1)] in methanol extracts from rat, sheep, human and dog hepatocytes (1 µM) incubated for 4 h at 37°C with ^{14}C-derquantel[(2)]

Metabolite	**Species**			
R*t* (min)	**Rat**	**Sheep**	**Human**	**Dog**
~2.9	6.32	5.52	6.23	17.20
~11.6	ND	ND	2.68	ND
12.1-13.1	ND	ND	ND	1.94
~14.1	ND	ND	ND	10.85
~14.9	ND	ND	ND	2.31
15.4-16.0	ND	ND	ND	4.51
~17.0	ND	ND	ND	1.91
19.9-20.9	ND	ND	ND	2.91
~21.5	ND	ND	ND	1.10
22.6-23.3	ND	1.73	ND	ND
23.4-24.5	7.76	4.23	1.00	ND
23.9-25.6	7.60	1.96	7.72	ND
25.6-27.1	20.86	17.41	5.02	4.07
27.4-28.8	2.04	3.43	1.69	2.36
30.5-30.8	6.18	2.21	3.36	9.35
32.6-32.9	1.83	ND	ND	1.61
33.1-34.1	1.77	ND	ND	0.92
36.9-37.1	5.31	4.07	9.06	4.94
~38.1	ND	1.31	ND	ND
~39.9	ND	3.31	ND	2.93
41.6-42.1	18.65	25.09	50.97	14.29
45.6-45.9	3.13	8.59	1.77	5.17
47.4-47.5	1.49	ND	ND	ND
51.4-51.8	4.33	2.53	3.54	3.08
53.5-53.9	3.41	1.52	4.32	1.58
53.4-56.6	ND	2.70	ND	ND
~64.1	1.36	8.73	ND	ND
Total (%)	92.04	94.34	94.68	95.71

NOTES: (1) Average of triplicate samples (Hennessy, 2006). (2) ^{14}C-2-Desoxoparaherquamide, at 1 µM, was incubated with each lot of hepatocytes at 37ºC for 4 h. ND = not detectable.

As with the microsome study, the hepatocyte preparations produced several metabolites of derquantel (see Table 3.11). The profiling data show that ^{14}C-2-desoxoparaherquamide was extensively metabolized by hepatocytes from rats, sheep and dogs. Derquantel was moderately metabolized by human hepatocytes. In addition to unchanged ^{14}C-derquantel, 26 radioactive components were detected in rat, sheep, dog or human hepatic incubations. After a 4-hour incubation, unchanged derquantel accounted for approximately 18.7, 25.1, 14.3 and 51.0% of radioactivity in rat, sheep, dog and human hepatic incubations, respectively. The major metabolites of derquantel, M1, M19/M20, M10 and M12, accounted for approximately 3.1, 20.9, 6.2 and 5.3% of the radioactivity for

the rat hepatocyte preparations; 8.6, 17.4, 2.2 and 4.1% of the radioactivity for the sheep hepatocytes; 5.2, 4.1, 9.4 and 4.9% of the radioactivity for the dog hepatocytes; and 1.8, 5.0, 3.4 and 9.1% of the radioactivity for the hepatocyte preparations from humans. M2 accounted for approximately 1.4 and 8.7% of the radioactivity in 4-hour rat and sheep hepatic incubations, respectively. M2 was not detected in the 4-hour hepatocyte incubation samples from dogs and humans. Conversely, M15 was detected in only the 4-hour dog hepatic incubations and accounted for approximately 10.9% of the radioactivity. Recovery of radioactivity was high for all species: >92% in the rat; >94% in sheep; >95% in dogs; and >94% in humans.

Paraherquamide (PHQ) was metabolized rapidly by microsomes from sheep, dogs, and gerbils. In sheep, 20% of the paraherquamide remained after a 1-hour incubation. Microsomal metabolism was even faster in dogs (10% remained after 30 minutes) and was fastest in gerbils (7% remained after just 15 minutes). PHQ and 8 metabolites were found in microsomal incubations from sheep and gerbils. In dogs, PHQ and 12 metabolites were found in microsomal incubations. M5 was absent from the radiochromatogram of dog microsomal incubations. The various metabolites were structurally identified in dogs, gerbils and sheep (Figure 2).

The *in vivo* metabolism of PHQ was also evaluated in sheep, dogs and gerbils (Aloysius *et al.*, 2008). Radiochromatograms show PHQ and 5 metabolites in sheep faeces, 5 metabolites but no PHQ in dog faeces, and PHQ and 6 metabolites in gerbil faeces. In urine, radiochromatograms reveal PHQ and 3 metabolites for sheep, 6 metabolites but no PHQ in dogs, and 4 metabolites and no PHQ in gerbils. In plasma, there were 5 metabolites and PHQ for sheep, 2 metabolites and PHQ for dogs, and 6 metabolites and PHQ for gerbils. The proposed metabolism of PHQ and formation of its major metabolites is provided in Figure 3.

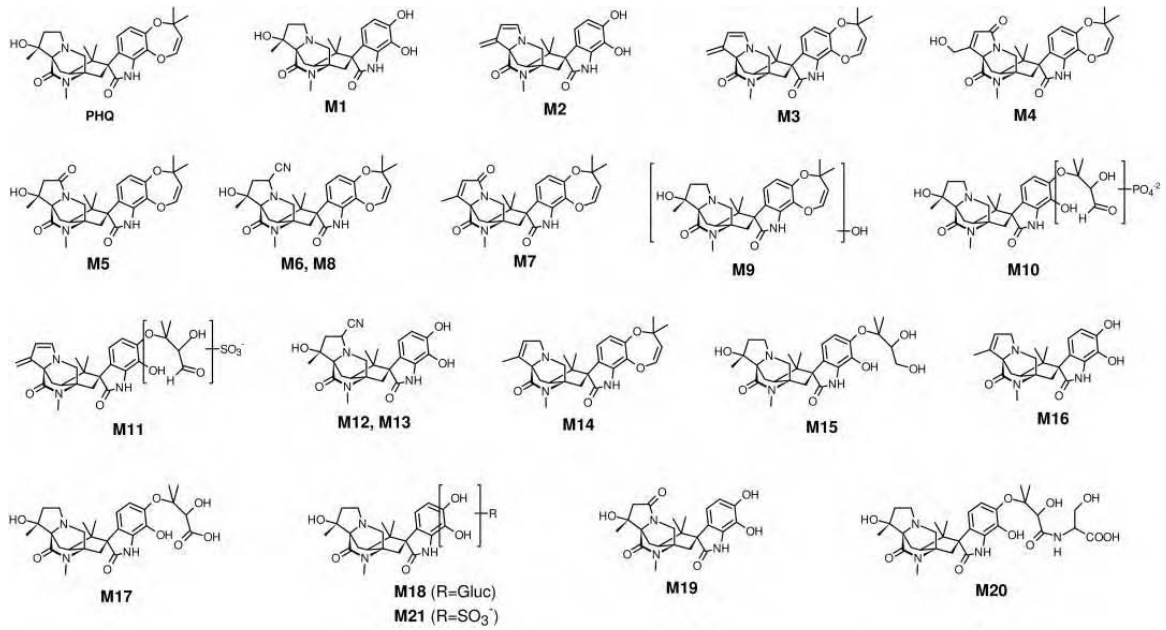


Figure 3.2. Identified metabolites in dogs, gerbils and sheep

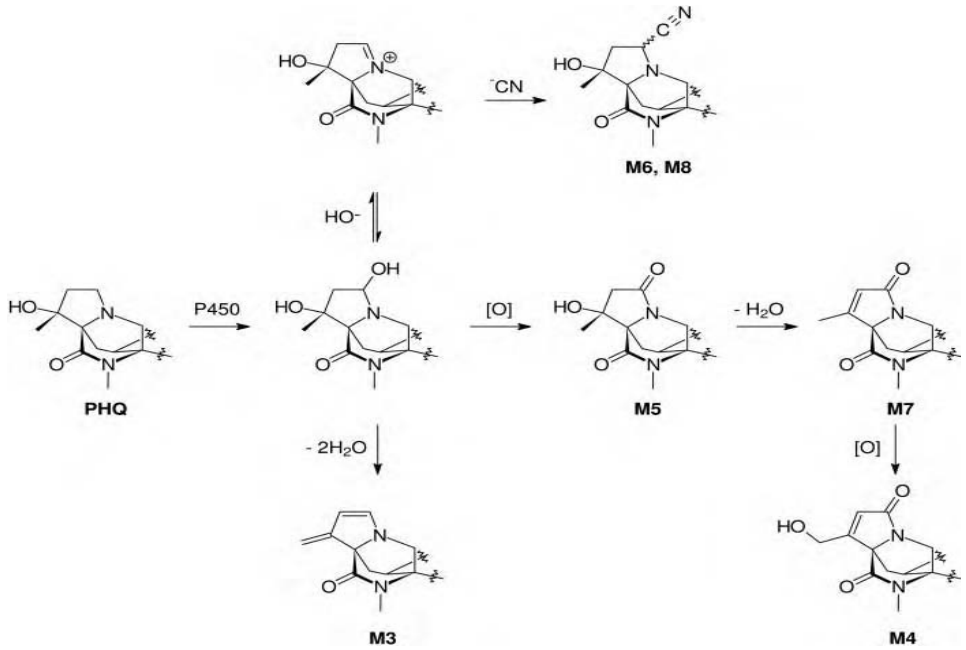


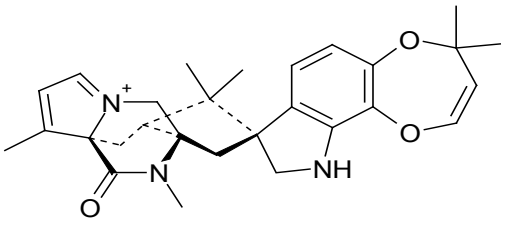
Figure 3.3. Proposed scheme for the metabolism of PHQ and formation of its major metabolites in gerbils, dogs and sheep

Metabolism in food-producing animals

Sheep

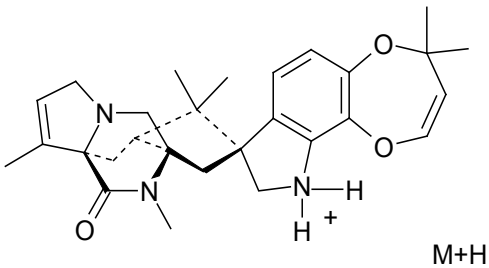
In a non-GLP-compliant study (Liu, 2009), tissues, urine, faeces and bile from sheep treated in the total residue study (Byrd and Liu, 2008) were evaluated for derquantel-related metabolites using LC-MS/MS. Although metabolism in sheep was the primary focus of the study, additional analyses were conducted using bile-cannulated rats to better identify metabolites in sheep. Tissue samples were extracted with saline/acetonitrile. Faecal samples were extracted with saline/acetonitrile or methanol/water. Urine and bile samples were centrifuged prior to analysis. Tissue and faecal extracts and urine and bile samples were separated using HPLC radiochromatography and chromatography effluents were collect at 0.25 minute intervals. Fractions were analysed using 96-well plates and the radioactivity in each fraction was determined with microscintillation and luminescence counting. Selected urine and bile samples and tissue and faecal extracts were analysed by LC-RAM/ESI-MS and MS/MS to obtain structural information on derquantel and its metabolites. Metabolite identification focused on compounds present in sheep liver. To facilitate metabolite identification, other sheep tissue extracts, urine, faeces and bile from both sheep and rats treated with derquantel were analysed. Parent derquantel was detected in sheep liver, muscle, fat, urine, faeces and bile.

Metabolite 1 was a major radioactive peak in the radiochromatograms of sheep liver eluting at approximately 44 minutes. It also was detected in sheep kidney, muscle, fat, urine, faeces and bile. LC-MS/MS spectral data of M1 in urine, liver and faeces yielded a $[M+H]^+$ at *m/z* 460. M1 was also found as a major metabolite in pooled rat bile collected from bile-cannulated rats treated orally with ^{14}C-derquantel. A degradation product, D1, resulting from the instability of M1 during isolation, was also detected. Further analysis resulted in the identification of D1 as reference standard KXN-869. M1 and D1 had been detected previously in *in vitro* studies using rat, sheep, dog and human liver microsomes and hepatocytes (Ma, 2006a, b).



MW = 460

Structure of M1

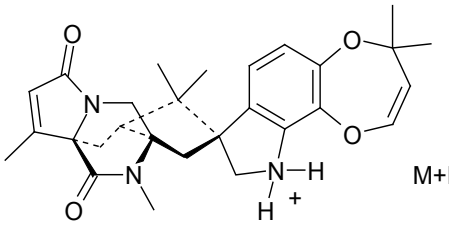


M+H = 462

Structure of D1

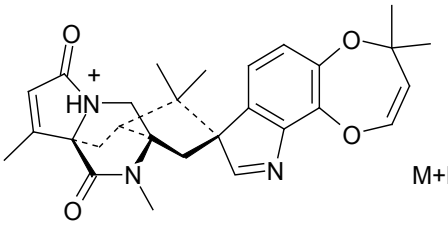
A second major radioactive peak, eluting at approximately 62 minutes, was found in sheep liver, kidney, muscle and fat. This metabolite, designated as M2, yielded a $[M+H]^+$ at *m/z* 476. M2 yielded $[M\text{-}d+D]^+$ at *m/z* 478 after H/D exchange, suggesting that M2 contains one exchangeable proton. M2 previously had been detected in *in vitro* studies using rat, sheep, dog and human liver microsomes and hepatocytes (Ma, 2006a, b).

Another radioactive peak, eluting at approximately 63–64 minutes, was found in sheep liver, kidney, muscle and fat. This metabolite, designated M6, yielded a $[M+H]^+$at *m/z* 474. M6 yielded a $[M\text{-}d+D]^+$ at *m/z* 476 after H/D exchange, suggesting that M6 contains two exchangeable protons. Metabolite M2 was partially converted to M6 during the isolation and purification process (Ma, 2006a, b).



M+H = 476

Structure of M2

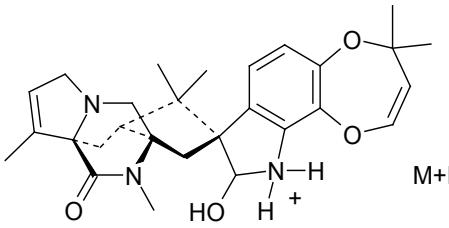


M+H = 474

Structure of M6

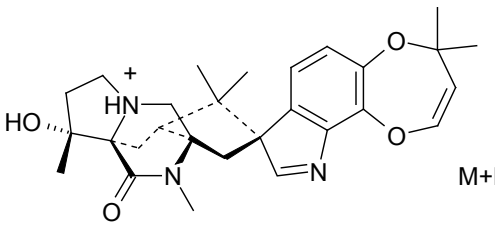
A major radioactive peak in muscle and fat, eluting at approximately 55 minutes, was also detected in liver and kidney. This metabolite, designated M5, yielded a $[M+H]^+$ at *m/z* 478. M5 previously had been detected in *in vitro* studies using rat, sheep, dog and human liver microsomes and hepatocytes but, in those studies, it was labelled as M14 (Ma, 2006a, b).

Another radioactive peak, eluting at approximately 37 minutes, was detected in sheep fat. This metabolite, designated M3, yielded a $[M+H]^+$ at *m/z* 478 and was similar to M5. M3 was also detected in *in vitro* metabolism studies using rat, sheep, dog and human liver microsomes and hepatocytes, but in those studies was designated M12 (Ma, 2006a, b).



M+H = 478

Structure of M5

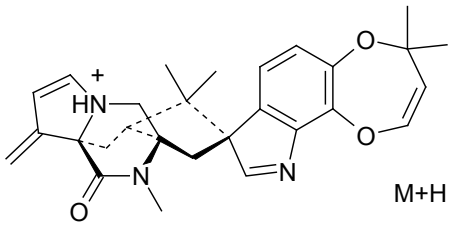


M+H = 478

Structure of M3

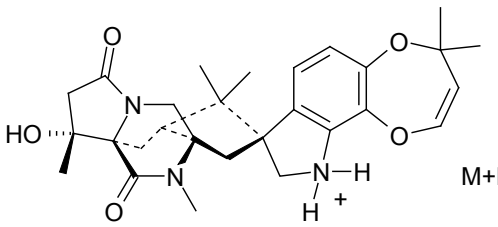
A radioactive peak, eluting at approximately 52.0 minutes, was detected in sheep fat, muscle, liver and kidney. This metabolite, designated M4, yielded a $[M+H]^+$ at *m/z* 458. M13, detected in *in vitro* metabolism studies using rat, sheep, dog and human liver microsomes and hepatocytes had the same $[M+H]^+$ at *m/z* 458 (Ma, 2006a, b).

A final radioactive peak, eluting at approximately 30.0 minutes, was detected in sheep faeces. This metabolite, designated M7, yielded a $[M+H]^+$ at *m/z* 494. M7 was also detected in *in vitro* metabolism studies using rat, sheep, dog and human liver microsomes and hepatocytes, but in those studies it was designated as M10 (Ma, 2006a, b).



M+H = 458

Structure of M4



M+H = 494

Structure of M7

A summary of the mass spectrometry (*m/z*) transitions (M + H) used to characterize derquantel and its metabolites is presented in Table 3.12.

Table 3.12. Derquantel metabolites identified in sheep tissue, bile, urine and faeces

Metabolite	[M+H]	Metabolite	[M+H]
M1	m/z 460	M5	m/z 478
M2	m/z 476	M6	m/z 474
M3	m/z 478	M7	m/z 494
M4	m/z 458	D1(KXN-869)	m/z 462

M1 and D1 were isolated for NMR and D1 was derived from M1 during isolation of M1.

The proposed metabolism scheme is shown in Figure 4. The bracketed structures indicate proposed intermediates.

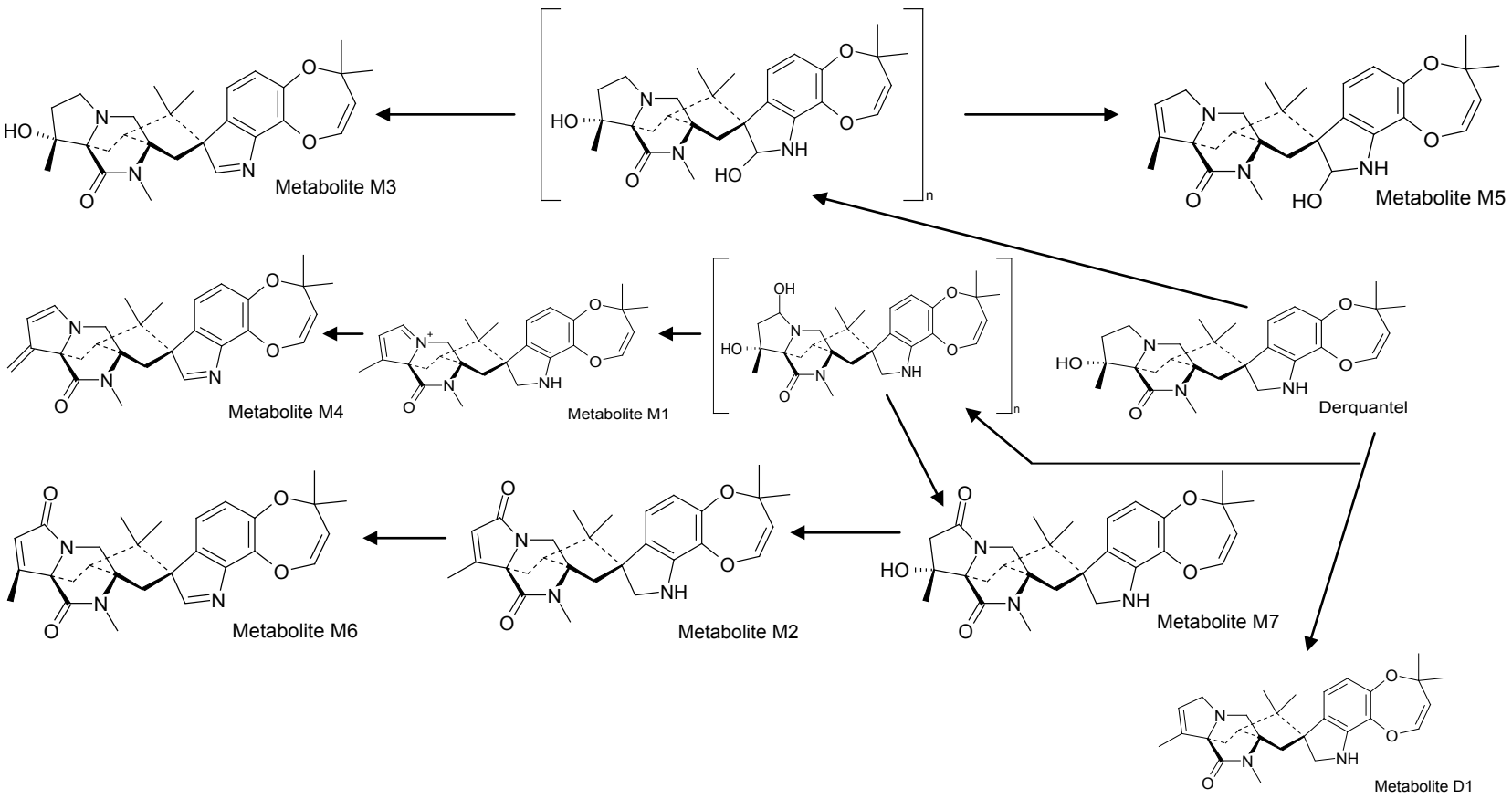


Figure 3.4. Proposed metabolism scheme in sheep. Bracketed structures indicate proposed intermediates.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabelled residue depletion studies

Sheep

One non-GLP- and two GLP-compliant studies were evaluated to assess total ^{14}C-derquantel residues (total radiolabelled residues - TRR) in sheep tissues.

In a non-GLP-compliant pilot disposition study (Byrd and Liu, 2006), sheep were treated orally *via* capsule with ^{14}C-derquantel to achieve a target dose of 2 mg derquantel/kg bw. The study was conducted in two phases. Phase 1 of the study (one female) required the use of 6 capsules to achieve the target dose due to the low specific activity of the test article. The radiopurity of the derquantel used in Phase 1 was approximately 90%. Phase 2 (four males; one per time point) used material with a higher specific activity and required only one capsule per sheep. The radiopurity of the derquantel used in Phase 2 was approximately 99%. The Phase 1 animal was slaughtered 3 h after dosing. The Phase 2 animals were slaughtered 6, 12, 24 and 48 h after dosing.

Although samples were available from one animal at 3 h withdrawal, data indicates that residues in tissues continue to increase until at least 6 h after dosing. The highest residues in each of the tissues were seen at the 6-hour sampling point. At all sampling times, total radiolabelled tissue residues were highest in liver. Residues also were high in fat and kidney, but declined more rapidly than did those in liver. Residues were consistently low in muscle. Results are summarized in Table 3.13.

Table 3.13. Total ^{14}C-derquantel equivalent residues (µg/kg) measured in tissues from sheep that received a single oral dose of 2 mg/kg bw

Group No.	Slaughter interval post-dosing	Liver	Kidney	Muscle	Fat
1	3 h	5133	611.4	122.7	351.8
2	6 h	6339	1461	420.5	1881
3	12 h	4549	983.6	267.1	838.0
4	24 h	2481	274.5	48.4	156.1
5	48 h	780.1	127.7	9.5	32.4

NOTES: The average value is reported for each specific time and tissue

The marker residue:total ratios were not specifically determined in this pilot study. In acetonitrile extracts of liver, derquantel represented 26.19%, 9.21% and 1.83% of the total radioactivity at 3, 12 and 24 h post-dosing. The acetonitrile extract from the 3-hour, 12-hour and 24-hour liver samples accounted for 76.0%, 79.5% and 58.6% of their TRR, respectively. In an acetonitrile extract of a 6-hour kidney sample, derquantel represented 26.29% of the total radioactivity. The acetonitrile extract from the 6-hour kidney sample accounted for 86.3% of the TRR.

In a GLP-compliant disposition study (Byrd and Liu, 2008), 20 crossbred sheep (3 per time point) were treated orally by gastric tube with ^{14}C-derquantel. Radiopurity was greater than 98.5% for all batches used. Animals received a single oral dose of approximately 2.10 mg derquantel/kg bw. Animals were slaughtered approximately 6, 12, 24, 48, 96 and 144 h after dosing. Samples were stored at below -10°C until analysed.

As in the pilot study, total radiolabelled residues were highest in liver and lowest in muscle. Residues in kidney and fat were similar at the early sampling times. At later sampling times, residues were generally higher in kidney than in fat. There was little change in concentrations between the 96- and 144-hour sampling times (See Table 3.14 and Figure 3.5).

The study also provided the data needed to assess the ratio of marker residue to total radioactivity (See Table 3.15).

Table 3.14. Total ^{14}C-derquantel equivalent residues (µg/kg) measured in tissues from sheep that received a single oral dose of 2 mg/kg bw

Group No.	Slaughter interval post-dosing)	Liver	Kidney	Muscle	Fat
1	6 h	5769	770	236	709
2	12 h	4155	549	131	438
3	24 h	3490	486	129	536
4	48 h	650	79	8	37
5	96 h	183	34	3	4
6	144 h	138	25	2	2

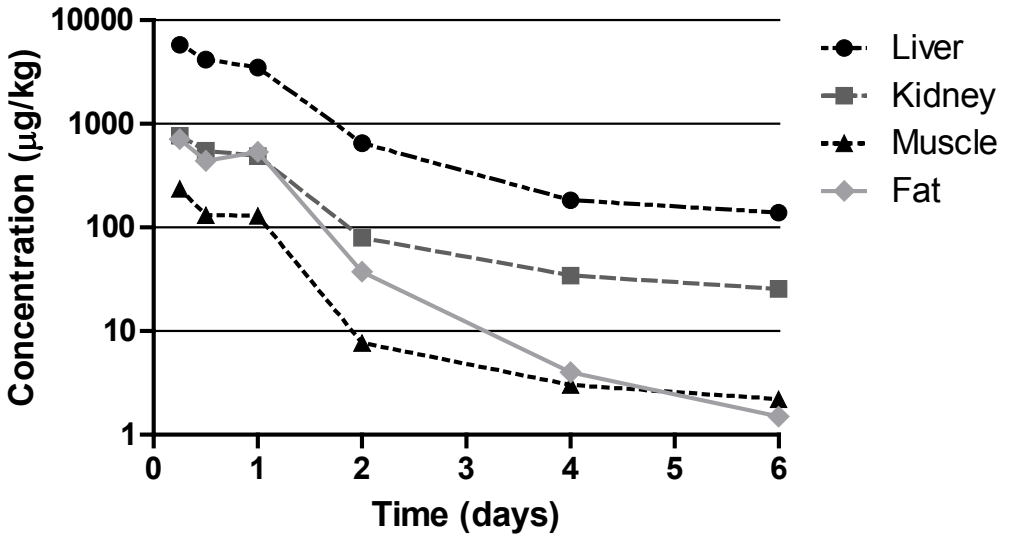


Figure 3.5. ^{14}C-derquantel equivalent residues (µg/kg) in sheep tissues

Table 3.15. Percentage of derquantel to total radiolabelled residues measured in tissues from sheep that received a single oral dose of 2 mg/kg bw

Group No.	Slaughter interval post-dosing	Liver (%)	Kidney (%)	Muscle (%)	Fat (%)
1	6 h	5.86	1.54	7.80	0.78
2	12 h	2.09	0.897	8.54	0.83
3	24 h	3.25	8.11	0.19	31.1
4	48 h	4.34	10.50	0	11.94
5	96 h	NA			
6	144 h	0			

NOTES: NA = not applicable.

In a second GLP-compliant study (Byrd, 2008), 6 sheep (one per time point) were treated once with 2 mg ^{14}C-2-deoxyparaherquamide/kg bw orally by gastric tube. The study was designed to confirm the marker to total ratio for derquantel in sheep. The test material had a radiopurity of approximately 92.5%. Animals were slaughtered 12, 24, 48, 96 and 672 h (0.5, 1, 2, 4 and 28 days) post-dose. Based on information in both the study protocol and study report, the samples were stored at below -70°C, significantly colder than in the previous GLP-compliant study. As in the pilot and previous GLP

studies, radiolabelled total residues were highest in liver and lowest in muscle. Residues in kidney were greater than residues in fat at all sampling times. Results are shown in Table 3.16 and Figure 3.6.

The study also was used to assess the ratio of marker residue to total radioactivity (See Table 3.17).

Table 3.16. Total ^{14}C-derquantel equivalent residues (µg/kg) measured in tissues from sheep that received a single oral dose of 2 mg/kg bw

Group No.	Slaughter interval post-dosing	Liver	Kidney	Muscle	Fat
1	12 h	2546	339	49.8	186
2	24 h	1254	145	17.8	114
3	48 h	551	66.0	6.7	23.7
4	96 h	229	53.7	3.4	13.3
5	672 h	17.4	6.2	0.7	2.2

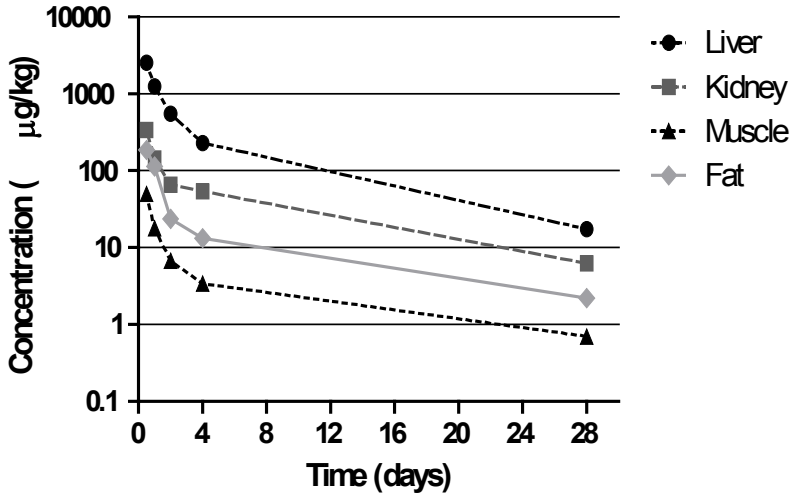


Figure 3.6. ^{14}C-derquantel equivalent residues (µg/kg) in sheep tissues

Table 3.17. Percentage of derquantel to total radiolabelled residues measured in tissues from sheep that received a single oral dose of 2 mg/kg bw

Group No.	Slaughter interval post-dosing	Liver (%)	Kidney (%)	Muscle (%)	Fat (%)
1	12 h	4.36	14.16	8.00	43.55
2	24 h	2.23	18.62	16.67	36.84
3	48 h	0.73	12.12	NA	16.67
4	96 h	0.44	0	NA	7.69
5	672 h	NA	NA	NA	NA

NOTES: NA = not analysed because of insufficient radioactivity to radioprofile

In samples collected from sheep, the TRR values are comparable between the two GLP-compliant studies, at time-points likely to be relevant to recommending an MRL, even though the samples in the two studies were stored at different temperatures (See Table 3.18).

Table 3.18. Total ^{14}C-derquantel equivalent residues (µg/kg) in tissues from two different GLP-compliant total residue studies where tissues were stored at different temperatures

Slaughter interval	Liver		Kidney		Muscle		Fat	
	Byrd and	Byrd,	Byrd and	Byrd,	Byrd and	Byrd,	Byrd and	Byrd,

post-dosing	Liu, 2008 < -10°C	2008 < -70°C	Liu, 2008 < -10°C	2008 < -70°C	Liu, 2008 < -10°C	2008 < -70°C	Liu, 2008 < -10°C	2008 < -70°C
6 h	5769		770		236		709	
12 h	4155	2546	549	339	131	49.8	438	186
24 h	3490	1254	486	145	129	17.8	536	114
48 h	650	551	79	66	8	6.7	37	23.7
96 h	183	229	34	53.7	3	3.4	4	13.3
144 h	138		25		2		2	
672 h		17.4		6.2		0.7		2.2

The marker residue:total residue ratios from the two radiolabelled residue studies are variable. The combined results for the two GLP-compliant studies are summarized in Table 3.19 (Gottschall, 2010b). Based on the significantly lower sample storage temperature in the Byrd. (2008) study, the marker residue:total residue ratios from this second GLP-compliant study, where samples were stored at < -70°C, may be a more accurate reflection of the marker:total ratio. However, the samples stored at < -10°C may represent a marker residue:total residue ratio more applicable to samples collected under normal use and monitoring conditions.

Table 3.19. Summary of marker:total (M/T) residue ratios (%) from the GLP-compliant total residue studies in sheep

Withdrawal time (hours)	Study ID	Derquantel Marker Residue:Total Residue (%)			
		Liver	Kidney	Muscle	Fat
6	1545N-60-05-171*	5.86	1.54	7.80	0.78
12	1545N-60-05-171	2.09	0.897	8.54	0.83
12	1545N-60-07-186**	4.36	14.16	8.00	43.55
24	1545N-60-05-171	3.25	8.11	0.19	31.1
24	1545N-60-07-186	2.23	18.62	16.67	36.84
48	1545N-60-05-171	4.34	10.50	0.0	11.94
48	1545N-60-07-186	0.73	12.12	NA	16.67
96	1545N-60-05-171	NA	NA	NA	NA
96	1545N-60-07-186	0.44	0.0	NA	7.69
144	1545N-60-05-171	0.0	NA	NA	NA
Mean M/T		2.5	6.8	5.8	15.0

NOTES: * = Storage at between -10°C and -20°C; 3 animals per time point (Byrd and Liu, 2008). ** = Storage at between -70°C and -80°C; 1 animal per time point (Byrd, 2008). NA = not analysed.

To evaluate storage stability, fortified tissue samples containing 1 or 500 µg derquantel/kg were analysed after storage at below -20°C for one month (Carnevale, 2008). Significant changes from the theoretical concentrations were evident in all tissues, indicating that derquantel fortified into tissues is not stable when stored at below -20°C (Table 3.20).

Table 3.20. Stability of derquantel in fortified tissues stored at below -20°C

Tissue	Theoretical concentration (µg/kg)	Concentration of Derquantel	% Change from theoretical	Mean % change from theoretical
Liver	1.00	0.47 ± 18.4	-53.0	-49.8
	500.0	266.9 ± 7.6	-46.6	
Kidney	1.00	0.27 ± 12.8	-73.0	-64.5
	500.0	219.8 ± 8.0	-56.0	
Muscle	1.00	0.2 ± 49.6	-80.0	-76.1
	500.0	139.7 ± 26.9	-72.1	
Fat	1.00	0.7 ± 7.7	-30.0	-37.4
	500.0	276.1 ± 10.3	-44.8	

As part of the validation for an ultraperformance liquid chromatographic (UPLC-MS/MS) procedure, an extended storage stability study was conducted using samples from the residue depletion study. Samples originally collected and analysed in Australia were shipped frozen to the method development laboratory. Samples were shipped, received and stored at below -70°C. The time from initial analysis to second analysis (re-analysis) was from 4 to 5 months, depending on the tissue. Results for the two analyses on the single overlapping analysis day (Sample day 4; 96 h withdrawal) compared favourably (Chambers, 2009). Results are shown in Table 3.21.

Table 3.21. Residues of derquantel (µg/kg) in sheep treated with 3 mg derquantel/kg bw and 0.3 mg abamectin/kg bw analysed at two different laboratories

Laboratory	Liver	Kidney	Muscle	Subcutaneous fat	Perirenal fat
Original*	2.98 ± 2.42	1.80 ± 1.54	<LOQ†	4.75 ± 8.47	3.99 ± 5.25
Re-analysis**	2.64 ± 1.65	1.27 ± 1.46	0.45 ± 0.52	4.46 ± 4.45	3.50 ± 4.12

NOTES: * LOQ = 1.0 µg/kg. ** LOQ = 0.1 µg/kg. † = Reported by laboratory as 0.51 ± 0.58 µg/kg.

When samples fortified with derquantel are stored at below -20°C, there is a loss of approximately 50% from theoretical after 1 month of storage. When samples containing incurred derquantel residues are stored at below -70°C, the nominal concentrations are consistent after 4 to 5 months of storage, even when analysed at two different facilities. Taken together, these two studies indicate that derquantel requires storage at significantly lower temperatures than those usually employed in residue studies or for monitoring samples.

Residue depletion studies with unlabelled drug

Sheep

In the only residue depletion study (Chambers, 2009) provided, equal numbers of females and male castrate Merino sheep (38) and 2_{nd} Cross Prime lambs (38), were randomly allocated to 12 slaughter groups (6 animals per group, with 4 controls). Animals in groups 2–13 were treated orally with the test formulation containing derquantel and abamectin. The dose delivered was 3 mg derquantel/kg bw and 0.3 mg abamectin/kg bw (150% of the label dose). Group 1 animals served as controls and received tap water orally.

Two control animals were slaughtered 6 h post treatment. At slaughter, the entire liver, both whole kidneys, gluteal muscle, perirenal and subcutaneous fat samples were collected. Tissues were weighed and diced, and frozen at approximately -70 to -80°C for 24 h. Further processing consisted of homogenization with dry ice to produce a fine powder. Additional gluteal muscle samples were collected, diced, and homogenized with a 0.9% saline solution. Samples were stored at -70 to -80°C pending analysis.

Analyses were conducted at two locations. Site 1 (original method; Table 3.22) analysed tissues from all animals for derquantel and abamectin in group order. Site 2 (UPLC-MS/MS; Table 3.23) re-analysed tissues for derquantel only, in reverse group order, and only for Groups 5 to 13 (96–840 h post-dosing). The LOQ for all tissues is 1.0 µg/kg at Analytical Site 1 and 0.1 µg/kg at Analytical Site 2. The study results are summarized in Tables 3.22 and 3.23.

No tissue residue depletion information was provided for chickens, turkeys, rabbits or residues in milk and eggs.

Table 3.22. Residues of derquantel (µg/kg) in tissues of sheep treated with the combination product to deliver 3 mg derquantel/kg bw and 0.3 mg abamectin/kg bw

Slaughter time post-dose	Liver	Kidney	Muscle	SC Fat	PR Fat
12 h	421.5 ± 131.7	211.3 ± 55.8	43.1 ± 29.0	720.9 ± 362.7	829.9 ± 340.2
24 h	247.9 ± 122.4	121.3 ± 49.1	24.7 ± 11.9	594.5 ± 297.2	461.8 ± 173.0
48 h	67.6 ± 60.8	22.3 ± 17.5	4.36 ± 4.86	125.5 ± 154.8	90.5 ± 61.2
96 h	2.98 ± 2.42	1.80 ± 1.54	<LOQ	4.75 ± 8.47	3.99 ± 5.25
144 h	<LOQ	<LOQ	<LOQ	1.93	<LOQ
192 h	<LOQ	<LOQ	<LOQ	1.08	<LOQ
240 h	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
336 h	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
408 h	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
504 h	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
672 h	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
840 h	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

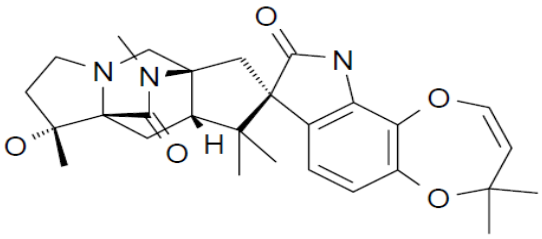
NOTES: LOQ = 1µg/kg (Analytical Site 1). SC Fat = subcutaneous fat; PR Fat = perirenal fat.

Table 3.23. Residues of derquantel (µg/kg) in tissues of sheep treated with the combination product to deliver 3 mg derquantel/kg bw and 0.3 mg abamectin/kg bw

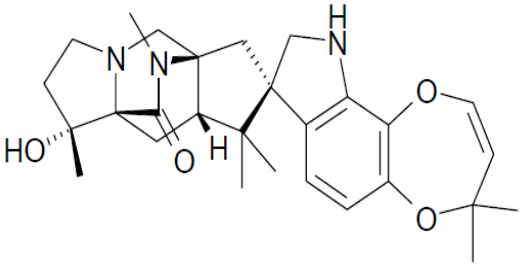
Slaughter time post-dose	Liver	Kidney	Muscle	SC Fat	PR Fat
12 h	NM	NM	NM	NM	NM
24 h	NM	NM	NM	NM	NM
48 h	NM	NM	NM	NM	NM
96 h	2.64 ± 1.65	1.27 ± 1.46	0.45 ± 0.52	4.46 ± 4.45	3.50 ± 4.12
144 h	0.90 ± 0.38	0.12	<LOQ	0.37 ± 0.43	0.31 ± 0.16
192 h	0.36 ± 0.16	0.14	<LOQ	0.39 ± 0.36	0.29 ± 0.21
240	0.67 ± 0.33	<LOQ	<LOQ	<LOQ	0.34 ± 0.40
336	0.36 ± 0.06	<LOQ	<LOQ	0.114	0.16 ± 0.03
408	0.31 ± 0.14	<LOQ	<LOQ	<LOQ	0.12 ± 0.02
504	0.20 ± 0.10	<LOQ	<LOQ	<LOQ	0.11 ± 0.01
672 h	0.32 ± 0.19	<LOQ	<LOQ	<LOQ	0.16 ± 0.03
840 h	0.19 ± 0.11	<LOQ	<LOQ	<LOQ	0.13

NOTES: LOQ = 0.1 µg/kg (Analytical Site 2). SC Fat = subcutaneous fat; PR Fat = perirenal fat; NM = not measured.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES



Paraherquamide/PF-03198957
Role: internal standard
Chemical Name: Paraherquamide
CAS Registry Number: 77392-58-6
Chemical Formula: $C_{28}H_{35}N_3O_5$
Molecular Weight: 493.6



2-DOPH(**Derquantel**)/PF-00520904
Role: active pharmacological compound
Chemical Name: 2-deoxoparaherquamide
CAS Registry Number: 187865-22-1
Chemical Formula: $C_{28}H_{37}N_3O_4$
Molecular Weight: 479.6

Two different analytical methods have been developed for the detection of derquantel; validation was performed against OECD GLP (EU/VICH and Aus/New Zealand/SA) regulations. These methods comply with the requirements of Australian Veterinary Medicines Authority (APVMA) Residue Guideline No. 26 – Veterinary Drug Residue Analytical Method, and "The Rules Governing Medicinal Products in the European Community – Volume VIII October 2005– Established by the European Community of Maximum Residue Limits (MRLs) for Residues of Veterinary Medicinal

Products in Foodstuffs of Animal Origin" as well as with applicable Pfizer Animal Health Standard Operating Procedures.

The two analytical methods are based on a liquid extraction of homogenized ovine tissue followed by a solid-phase extraction (SPE) prior to reconstitution and quantitation by Liquid Chromatography/Mass Spectrometry (LC/MS). The most significant differences between the two methods are the treatment of muscle samples, the use (or not) of high-resolution chromatography for separation, and MS/MS (versus MS) for detection. The original method muscle assay used a saline homogenate and a matrix standard curve, whereas the revised method muscle assay used cryogenically processed muscle and a neat (matrix free) solution standard curve. All other tissues in both assays used cryogenically processed samples and neat solution standard curves. The original muscle method required these modifications because of significant ion enhancement during the electrospray ionization (ESI) process observed during method development. The original method is based on a conventional chromatographic separation followed by a single dimensional mass spectrometry characterization (MS^1 on a single quadrupole), whereas the revised method used fast and high resolution chromatography coupled to multi-dimensional mass spectrometry (MS^2 on a triple quadrupole) (See Guyton, 2009). The LC-MS system used by the original method is quantitative to 1 µg/kg whereas the UPLC-MS/MS method used by the revised method is quantitative to 0.1 µg/kg.

Original method

Blank liver, kidney and fat samples are prepared cryogenically as a powder homogenate. To prepare a homogenate, intact frozen tissue is sliced into thin sections and stored at -15ºC prior to homogenization. An approximately equivalent amount of dry ice is added to the tissue (1:1 v/v*)* and the mixture is transferred to a blending homogenizer and processed to a fine powder. Blank muscle is prepared as a saline homogenate. To prepare a homogenate, each intact tissue is sliced into wafer thin shavings and chilled physiological saline is added to the muscle tissue sections at a ratio of 2 parts tissue to 1 part saline. The sample is contained in an ice bath and homogenized to a smooth consistency using a homogenizer. All sample processing is performed in small batches to avoid unnecessary time that samples are exposed to temperatures above -80°C. Post processing, muscle homogenate is immediately transferred to -80°C storage. The tissue sample is fortified with the internal standard solution. The sample is left in ambient air for 15 min prior to the extraction with acetonitrile. After the addition of phosphate buffer, the extract is applied onto an MCX SPE cartridge. The column is eluted with acetonitrile/purified water (80/20, 6% NH_4OH) solution. After evaporation, and reconstitution with acetonitrile and purified water, 5.0 µl of the sample is injected onto the column (Xterra® MS C18 5 µm 2.1 × 150 mm stainless steel column) for detection and analysis mass spectrometry (single quadrupole mass spectrometer detector). The mobile phase consists of methanol (0.075% formic acid) : purified water (0.075% formic acid) [36:64]. The analytes elutes under isocratic conditions at a flow rate of 0.25 ml/min. The chromatographic run time is 10 min with the target analyte eluting at approximately 6 min and the internal standard eluting at approximately 7.5 min. Positive electrospray (+ESI) is used to ionize target analytes. Signals are recorded using the selected ion monitoring (SIM) mode; *m/z* 480 and *m/z* 494 are monitored to characterize 2-DOPH and its internal standard, respectively. The quantity of 2-DOPH in each sample is calculated from standard curves based on linear least squares of the respective peak area response ratios of 2-DOPH and its internal standard. There is no weighting applied and calibration curves are not forced through zero. Curve 'fit' is accepted where the coefficient of determination (r^2) is >0.995.

Revised method

Tissue samples are extracted with acetonitrile, followed by sample clean-up with cation exchange solid phase extraction (MCX-SPE). The eluant from the SPE column is evaporated to dryness and reconstituted with 20% acetonitrile in 10 mM ammonium acetate prior to UPLC-MS/MS analysis. The analytes are eluted from the UPLC column by increasing the mobile phase B composition (0.1% ammonium hydroxide in methanol) linearly from 20% to 80% over 0.5 min (mobile phase A being 0.1% ammonium hydroxide in water), with a return to initial conditions at 1.75 min and holding at 20% Mobile Phase B until the end of the 2 min run. Injection volume is set at 5.0 µl, sample temperature is kept at 4°C, and column (UPLC BEH C18, 1.7 µm, 2.1 × 50 mm) is maintained at

40°C. Electrospray ionization is preferred to other atmospheric pressure ionization techniques, and detection is conducted in the negative ion mode using acquisition of ions in the selected reaction monitoring (SRM) mode. 2-DOPH and paraherquamide transitions are *m/z* 480→405 and *m/z* 494→419, respectively. An internal standard calibration method is used for quantitation with paraherquamide as the internal standard. The plot of peak area ratio vs 2-DOPH concentration produces a line of best fit according to the chosen regression and weighting factor. For this validation the standard curves are fitted to a linear equation (y = mx+b), where *m* is the slope of the line and *b* is the y-intercept. The weighting factor used is 1/x. The concentrations are determined by back calculating against the fitted line from the peak area ratio. If back-calculated standards are more than ±15% of their nominal concentration, that standard is dropped from the regression.

SPECIFICITY AND SELECTIVITY

The specificity is assessed by monitoring a large set on blank tissues; no response is detected in the retention time region of interest.

Original method

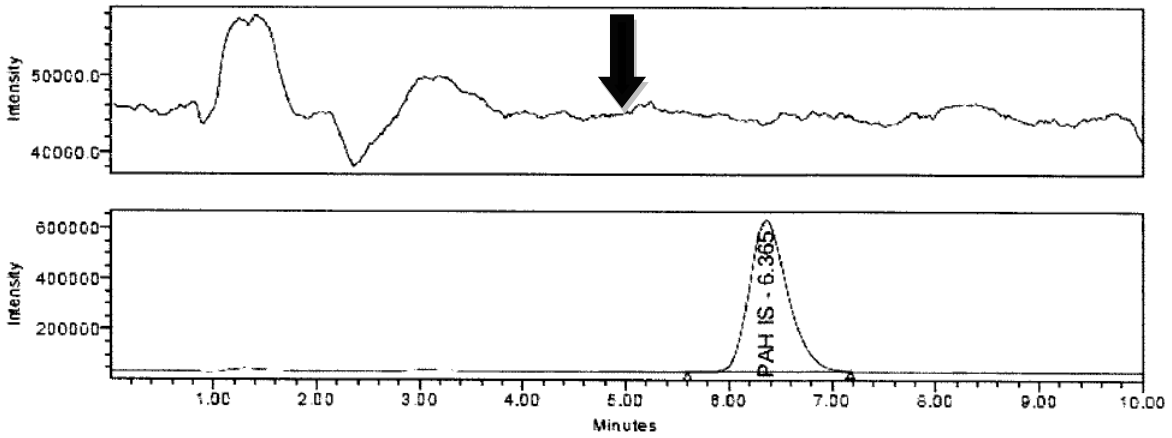


Figure 3.7. Example of an LC-MS trace of blank liver sample (DOPH at RT 4.98 min, internal standard at 6.37 min)

Figure 3.8. Example of an LC-MS trace of quality control (QC) liver sample fortified with 5 µg/kg with 2-DOPH (top trace) and the internal standard (bottom trace)

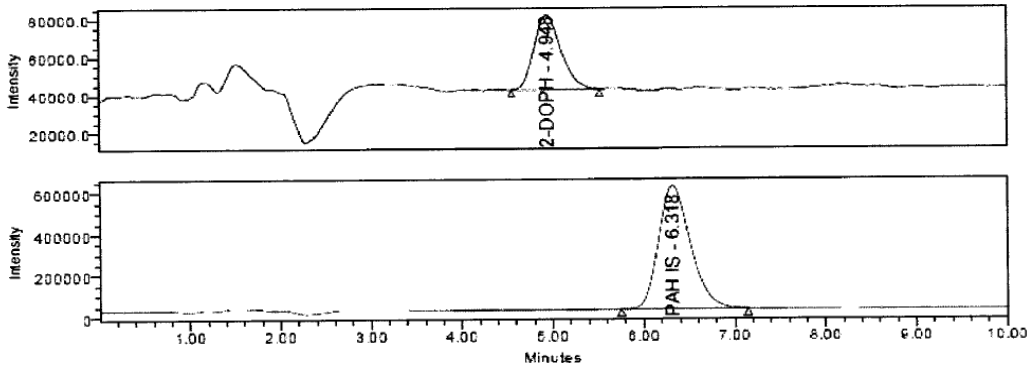
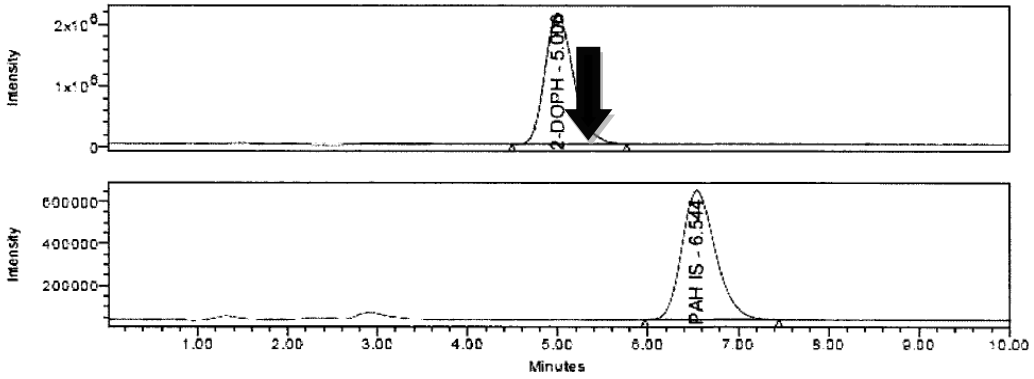


Figure 3.9. Example of an LC-MS trace of incurred residues in liver from animal 1336 (assay 1, 24 June 2008); 2-DOPH (top trace) and the internal standard (bottom trace)



Revised method

2-DOPH transitions *m/z* 480 →405 and *m/z* 494→419 for paraherquamide are not interfered with by abamectin under the chromatographic conditions employed and the mobile phase used to elute the target analyte and its internal standard, respectively.

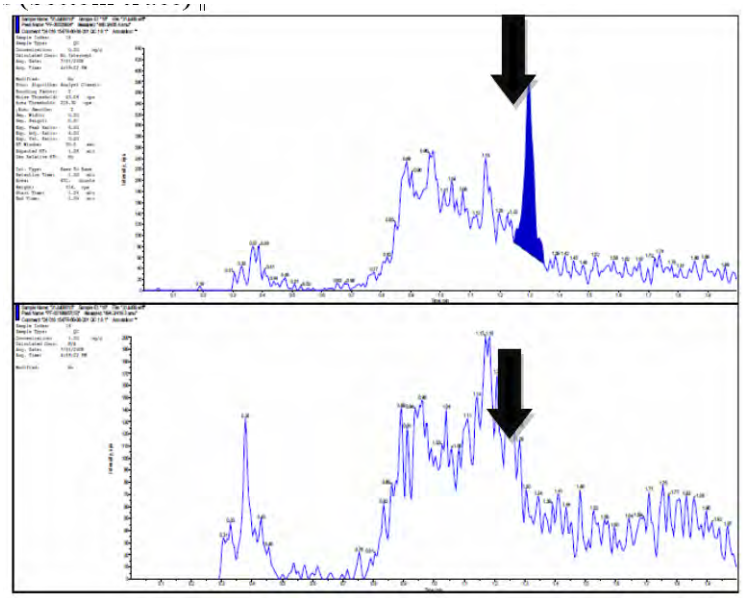


Figure 3.10. Example of an LC-MS/MS trace of blank liver sample; 2-DOPH (top trace) and the internal standard (bottom trace)

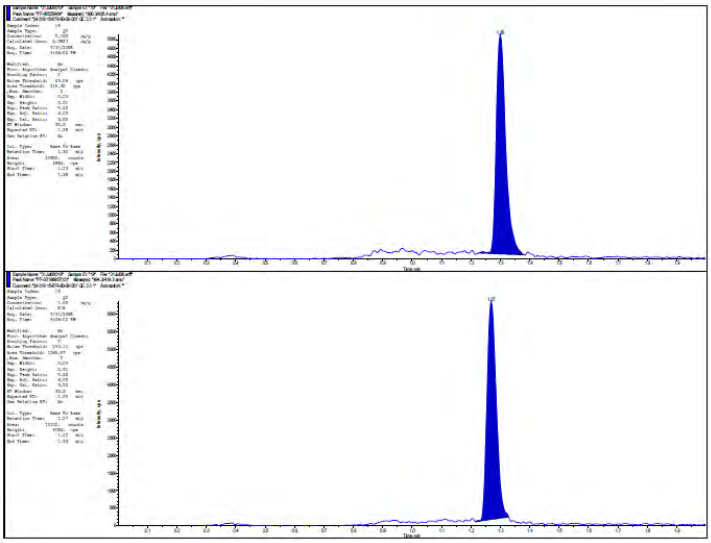


Figure 3.11. Example of an LC-MS/MS trace of quality control (QC) liver sample fortified with 0.1 µg/kg with 2-DOPH (top trace) and the internal standard (bottom trace)

ACCURACY AND PRECISION

Original method

The data in the validation study provided a fit for purpose accuracy and precision over the concentrations tested (1–500 µg/kg) for 2-DOPH quantitation in ovine tissue. An ad hoc linearity (r^2 >0.998) is demonstrated; intra-day accuracy of the validation samples is in the ranges 84.3–99.7% (liver), 88.6-103.6% (kidney), 72.3-117.7% (muscle) and 88.4-111.2% (fat). The imprecision (%CV) for each tissue is in the ranges of 2.4-6.9% (liver), 3.2-11.5% (kidney), 3.2-24.0% (muscle) and 2.3-29.3% (fat). The inter-day accuracy is 86.1-108.6% across all tissue types and validation concentrations. The inter-day imprecision is 3.6-25.0% (but generally <15%) across all tissue types and validation concentrations.

Revised method

The analysis of the provided validation data confirms very good accuracy and precision for 2-DOPH quantitation over the concentrations tested (0.1 to 1 µg/kg) in ovine tissue. An ad hoc linearity (r^2 >0.990) is shown; intra-day accuracy of the validation samples was in the ranges 52.0-139.0% (liver), 48.4-113.6% (kidney), 58.4-92.9% (muscle) and 73.8-123.0% (fat). The imprecision (CV%) for each tissue was in the ranges 8.5-16.9% (liver), 4.6-19.9% (kidney), 4.3-11.3% (muscle) and 3.5-6.8% (fat). The inter-day accuracy is 68.3-102.3% across all tissue types and validation concentrations. The inter-day imprecision is 5.5-23.6% (but generally, <20%) across all tissue types and validation concentrations.

LODs and LOQs

Determinations of LODs and LOQs for 2-DOPH in ovine tissue are made from concentration responses from 20 ovine tissue blanks per matrix and from 6 different sources per matrix. The LOD is calculated considering the mean concentration response (at the retention time of 2-DOPH) in the 20 blank samples plus three standard deviation of the mean value. The original method LOD is 0.04-0.07 µg/kg. The revised method LOD is 0.007-0.022 µg/kg. The limits of quantitation are set at the lowest concentration used for the validation runs. For the original method, the LOQ is 1.0 µg/kg, whereas, for the revised method, the LOQ is 0.1 µg/kg.

Table 3.24. Summary of performance for 2-DOPH in different matrices (Original method)

Assay parameter	Liver	Kidney	Muscle	Fat
Linearity r^2	>0.998	>0.999	>0.999	>0.999
Estimated LOD (µg/kg)	0.07	0.05	0.04	0.05
LOQ (µg/kg)	1.0	1.0	1.0	1.0
Interday Accuracy ± Imprecision (%)				
1.0 µg/kg	89.6 ± 5.1	98.7 ± 8.5	108.6 ± 15.6	100.3 ± 17.7
5.0 µg/kg	91.8 ± 4.9	91.9 ± 8.6	87.9 ± 25.0	91.1 ± 3.6
50 µg/kg	94.4 ± 6.5	93.3 ± 7.8	89.5 ± 11.8	98.3 ± 6.5
500 µg/kg	86.1 ± 3.9	88.7 ± 6.3	88.6 ± 6.4	91.4 ± 4.0
Absolute Recovery (%)				
1.0 µg/kg	57.1	73.3	186.7	81.0
5.0 µg/kg	57.8	60.7	70.7	90.0
50 µg/kg	64.9	100.0	53.6	89.2
500 µg/kg	68.6	64.8	58.4	93.3
Mean Abs Recovery (%)	62.1	74.7	92.4	88.4
Int. Std Recovery	56.1-85.6	66.3-108.2	41.0-94.6	72.0-104.7

Table 3.25. Summary of performance for 2-DOPH in different matrices (Revised method)

Assay Parameter	Liver	Kidney	Muscle	Fat
Linearity r^2	>0.996	>0.993	>0.990	>0.995
Estimated LOD (µg/kg)	0.007	0.009	0.022	0.020
LOQ (µg/kg)	0.1	0.1	0.1	0.1
Interday Accuracy ± Imprecision (%)				
0.1 µg/kg	91.9 ± 19.1	80.2 ± 18.9	75.5 ± 11.5	101.8 ± 17.2
0.5 µg/kg	102.3 ± 16.7	78.4 ± 23.6	72.0 ± 11.2	97.0 ± 6.4
1.0 µg/kg	82.2 ± 19.0	74.7 ± 17.5	68.3 ± 9.8	92.0 ± 5.5

STABILITY

The stability of the fortified tissue samples at -20°C was assessed and the results indicate that significant degradation of 2-DOPH residues (compared with freshly prepared samples) occurs upon extended frozen storage. An additional stability assessment concluded that 2-DOPH in ovine tissues is stable over the typical duration of an assay (including all steps of the analytical method, from sample preparation to injection into the autosampler).

The consequence of freeze-thaw cycles was investigated and assessed regarding the degradation of tissue samples. Results indicate that increasing the number of freeze-thaw cycles for incurred tissue samples or storing incurred samples at ≥ -20°C leads to a significant degradation of analyte in the matrix. 2-DOPH residues in the ovine matrix are most stable when stored at approximately -80°C and subjected to minimal freeze-thaw cycles. However, the mean 2-DOPH concentrations (µg/kg) in ovine muscle homogenate following extended storage stability at approximately -80°C over 3 and 6 months was outside the specified ±15% limits for most samples.

APPRAISAL

Derquantel has not been previously reviewed by the Committee. Derquantel, a spiroindole, is an oral anthelmintic for use in sheep. It is registered only as a combination product with abamectin. The combination product contains 10 mg/ml derquantel and 1 mg/ml abamectin. Derquantel, in combination with abamectin, is used to treat and control a broad range of adult and immature (L4) gastrointestinal nematodes of sheep. In the registered combination product, the withdrawal period is determined by the depletion of residues of the abamectin component. The 14-day withdrawal period identified on the registered labelling from New Zealand and the Republic of South Africa is consistent with the withdrawal period registered for abamectin alone in these countries and in Australia.

Derquantel is metabolized extensively; there are 8 principle metabolites in sheep. The metabolic profiles are qualitatively similar across the tested species. In studies using preparations of hepatocytes and microsomes, more than 20 metabolites were identified in test animals and sheep. Derquantel is eliminated relatively rapidly, primarily in the faeces. The terminal plasma half-life is approximately 10 h.

Depletion studies using radiolabelled derquantel were conducted in the sheep, the only species for which derquantel is registered. Radiolabelled total residues in muscle were uniformly low. At 6 h withdrawal, radiolabelled residues were highest in liver, followed by kidney and fat. Residues decline rapidly over the next 24–48 h, but were still detectable in one study in all tissues at 28 days withdrawal. There are no residue data available for sheep milk.

Data from two total residue studies are available for determining the marker to total ratio for derquantel in sheep tissues. The first study used a large number of animals, but samples were stored only at < -10°C, a temperature subsequently determined to be insufficient for maintaining derquantel stability during storage. The second study, intended as a confirmatory study, utilized a much small number of animals but samples were stored at < -70°C, a temperature demonstrated to maintain derquantel stability in tissues. Effectively, neither study is ideal because the marker to total residue ratios are variable. As such, the marker to total residue ratios from both studies are used when estimating exposures.

Derquantel, although constituting a small percentage of the total residues, is an appropriate marker residue for derquantel residues in tissues.

A single residue depletion study using unlabelled derquantel was evaluated (as the commercial combination product formulation) in the target species, sheep. The dose was 150% of the labelled dose for sheep (3 mg/kg bw derquantel and 0.3 mg abamectin /kg bw). As in the radiolabelled derquantel studies, residues in muscle were uniformly low. In this residue depletion study, the highest concentrations of derquantel residues at early sampling times were found in fat, followed by liver and kidney. At later sampling times, the highest residues were found in liver. Using an analytical method with an LOQ of 1 µg/kg, derquantel residues were below the LOQ at 8 days post-treatment in all tissues except subcutaneous fat. When the same samples were re-analysed using an analytical method with an LOQ of 0.1 µg/kg, derquantel residues were still quantifiable in liver and perirenal fat at 35 days withdrawal, the final time point in the study. Because derquantel is not registered for use in lactating sheep, there are no residue data for sheep milk.

The MRLs recommended for sheep liver and fat are based on the upper limit of the one-sided 95% confidence interval over the 95th percentile (“95/95 tolerance limit”) for the 8-day post-treatment data from the unlabelled residue depletion study. The MRLs recommended for sheep muscle and kidney are twice the LOQ of the UPLC-MS/MS method. No MRL is recommended for sheep milk as no residue data were provided for milk.

MAXIMUM RESIDUE LIMITS

In recommending MRLs for derquantel, the Committee considered the following factors:

- An ADI of 0–0.3 µg/kg of body weight was established by the Committee based on an acute toxicological endpoint. This ADI is equivalent to up to 18 µg for a 60 kg person.

- Derquantel is extensively metabolized; derquantel represents, conservatively, 6% of total residues in muscle, 3% in liver, 7% in kidney and 15% in fat. Derquantel, although constituting a small percentage of total residues, is suitable as the marker residue in tissues. No data are provided for sheep milk.
- Liver contains the highest concentration of total radiolabelled residues at all sampling times. Fat contains the highest concentrations of derquantel residues in the unlabelled residue depletion studies at early sampling points. At times beyond the 4-day sampling time, residues are highest in liver. The highest concentration of the proposed marker residue, derquantel, at time points relevant to recommending MRLs is found in liver, followed by fat, then kidney and then muscle. Liver and fat can serve as the target tissues.
- A validated analytical procedure for the determination of derquantel in edible sheep tissues (liver, kidney, muscle and fat) is available and may be used for monitoring purposes.
- The MRLs recommended for sheep liver and fat are based on the upper limit of the one-sided 95% confidence interval over the 95th percentile ("95/95 tolerance limit") for the 8-day post-treatment data from the unlabelled residue depletion study. The MRLs recommended for sheep muscle and kidney are twice the LOQ of the UPLC-MS/MS method.
- No MRLs were recommended for sheep milk as no residue data were provided for milk.

The Committee recommended MRLs for derquantel in sheep tissues of 0.2 µg/kg in muscle, 2.0 µg/kg in liver, 0.2 µg/kg in kidney and 0.7 µg/kg in fat, determined as derquantel. No MRLs are recommended for sheep milk.

Using the model diet and the ratio of the concentration of the marker residue to the concentration of the total residues noted above, these MRLs result in an intake of 8 µg/person, which represents 45% of the upper bound of the ADI.

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Monensin

First draft prepared by
Bruno Le Bizec, Nantes, France
and
Pascal Sanders, Fougères, France

Addendum to the monograph prepared by the 70th meeting of the Committee
and published in FAO JECFA Monograph 6

IDENTITY

International Non-proprietary Name(s) (INN): Monensin sodium

Synonyms: Monensin A sodium salt; Monensin sodium; Monensin sodium salt; NSC 343257; Sodium monensin; Elancoban®; Elancogran®, Coban®, Rumensin®, Coxidin®

IUPAC Names: Stereoisomer of 2-[2-ethyloctahydro-3′methyl-5′[tetrahydro-6-hydroxy-6-(hydroxymethyl)]-3,5-dimethyl-2Hpyran-2-yl] [2,2′-bifuran′5′yl]]-9-hydroxy-β-methoxy-a,γ,2,8,-tetramethyl-1,6-dioxaspiro[4.5]decan-7-butanoic acid.

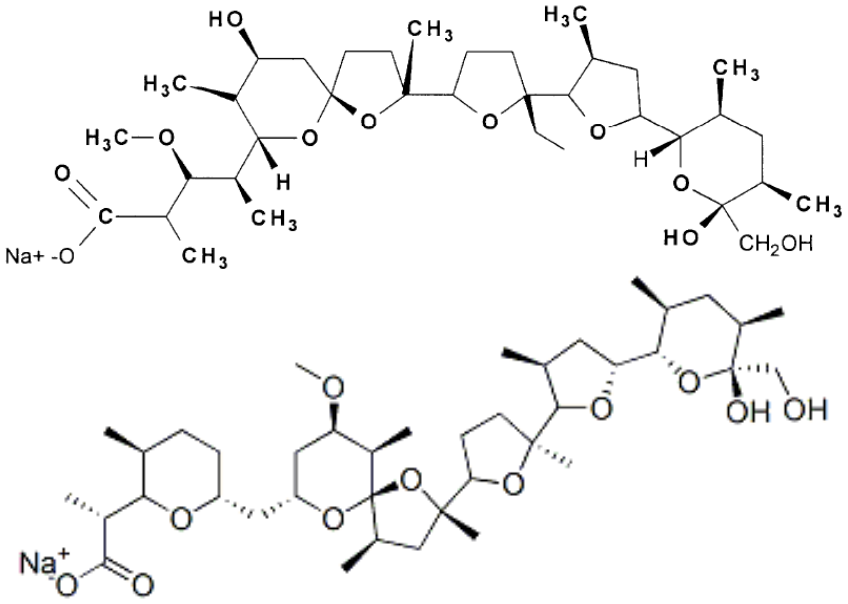
and: 4-[2-[5-ethyl-5-[5-[6-hydroxy-6-(hydroxymethyl)-3,5-dimethyl-oxan-2-yl]-3-methyl-oxolan-2-yl]oxolan-2-yl]-9-hydroxy-2,8-dimethyl-1,6-dioxaspiro[4.5]dec-7-yl]-3-methoxy-2-methyl-pentanoic acid

Chemical Abstracts Service Number: Monensin 17090-78-8; Monensin Sodium 22373-78-0

Molecular Formula: Monensin A $C_{36}H_{61}O_{11}Na$

Molecular Mass: 693 g/mol

Chemical structures: Monensin A as sodium salt (upper); nigericin as sodium salt used as internal standard (lower).



BACKGROUND

The Committee evaluated the residue safety of monensin in different species of food animals at its 70th meeting (FAO/WHO, 2009). In the evaluation, the Committee considered Monensin A (shown above) to be a suitable marker residue for monensin in milk and tissues in all species. At the 18th meeting of the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF), draft maximum residue limits (MRLs) for monensin in various species as recommended by the 70th meeting of JECFA were proposed for Step 5/8 of the evaluation procedure. The Committee recommended permanent MRLs for monensin in poultry (chicken, turkey and quail) tissues of 10 μg/kg in liver, kidney and muscle, and 100 μg/kg in fat. The Committee recommended permanent MRLs for monensin in ruminant (cattle, sheep and goat) tissues of 10 μg/kg in kidney and muscle, 20 μg/kg in liver, 100 μg/kg in fat and 2 μg/kg in milk. The original assessment of the MRL was based on a limited dataset (Bassissi and Larvor, 2007; Bagg and Dick, 1999, 2000); much of the residue data were below the limit of quantitation (50 μg/kg or 25 μg/kg, depending on the method used). In consequence, a re-evaluation of the monensin MRL in cattle liver was requested and monensin was added to the priority list of veterinary drugs for evaluation or re-evaluation of the cattle liver MRL by JECFA.

The sponsor submitted additional data and requested that the new data and one previously submitted study be considered in conjunction with that of Bassissi and Larvor (2007). MacDougall and Roberts published in 2011 a paper indicating that the Codex MRL (20 μg/kg) would not be compatible with a zero time withdrawal period. Elanco has submitted a variation of the EU MRLs requesting an increase in the MRL for bovine liver from 30 μg/kg to 50 μg/kg and for kidney from 2 μg/kg to 10 μg/kg.

The sponsor submitted four main documents in support of the expected additional data for monensin in cattle tissues and milk. The first was a copy of the method formatted according to the ISO 78/2 format (Analytical Method 1775 ISO 78/2). This is an improved method for the determination and confirmation of marker residue (monensin A) in cattle tissues. The second document is a report for a GLP-compliant validation study conducted for monensin A in cattle tissues and milk (Bassissi and Larvor, 2007). The third document is a validation data of an analytical method for the determination of Monensin A in bovine liver, kidney, muscle, fat and milk by LC-MS/MS (MacDougall, 2011). The fourth document (presented in three volumes) corresponds to a non-clinical laboratory study (GLP) on tissue and milk residue in dairy cows following a single oral dose administration of monensin controlled release capsule (MacDougall and Roberts, 2011). Monensin is approved for administration to cattle in a variety of formulations, and including in particular an intra-ruminal controlled release capsule (CRC).

The sponsor requests that any proposed changes to the cattle liver MRL be extended to other ruminants (i.e. goats and sheep) as well.

APPROVED USES FOR MONENSIN IN CATTLE

The sponsor clarified the currently labelled uses for monensin. Canada, USA, Australia and New Zealand have label uses for monensin that could result in a monensin dose that exceeds 1 mg/kg bw, and under some circumstances be as high as 2 mg/kg bw.

Monensin Capsule

The monensin CRC is a winged plastic delivery device for cattle which is introduced into the rumen via the mouth. It contains a stack of monensin tablets (total of 32 g of monensin) which slowly release monensin into the rumen via an orifice in the device. In most countries where the CRC is registered, the label stipulates a 200 kg minimum body weight for cattle that can be dosed with the capsule. This means that at the start of the treatment period, these 200-kg cattle would be receiving 320 mg monensin/head/day or 1.6 mg/kg bw/day (based on an average payout period of 100 days). In Canada, differences in beef cattle breeds and diet has resulted in a labelled average capsule payout period of 80

days. Thus the dose for 200-kg beef cattle in Canada is almost 2 mg monensin/kg bw/day at the start of the treatment period.

In New Zealand, the CRC label has a minimum weight of 300 kg (to account for the smaller frames of Jersey cattle, a common dairying breed in New Zealand); this still delivers a monensin dose of 1.07 mg monensin/kg bw/day.

On the Australian CRC label, the restriction has been removed, allowing the capsule to be re-dosed at less than 100 days. The purpose of the removal of this restriction and the removal of other restrictions on some labels for concurrent use (see below) was to ensure continuous protection of cattle from bloat and ketosis during periods of high challenge. This means that cattle dosed with a monensin CRC, could be provided with a second CRC, at the same time as the first dose is completing its payout period, resulting in a short time where cattle could be getting twice the usual dose, or 640 mg monensin/head/day, or up to 2 mg monensin/kg bw/day.

CONCURRENT USE OF MONENSIN CAPSULE AND MONENSIN PREMIX

In New Zealand, the monensin CRC label permits cattle in excess of 600 kg to receive additional monensin product (according to the New Zealand label for other monensin products, the additional dose would be 300 mg monensin/head/day). Thus a 600 kg animal could receive 620 mg monensin/head/day or 1.03 mg monensin/kg bw/day.

In Canada, concurrent dosing of the monensin CRC with monensin premix is also permitted for dairy cattle. Thus, assuming a minimum weight of 550 kg for a Canadian dairy cow and an upper feed intake of 4.5% bw per day, concurrent use of monensin (95-day payout period for Canadian dairy cattle) and 396 mg monensin/head/day from the feed (16 ppm in the feed – upper limit). The total dose of monensin would be 1.3 mg monensin/kg bw/day.

The Australian CRC label also has no restrictions preventing concurrent use with in-feed monensin premix.

Monensin premix

In Canada, mature Holstein dairy cattle weigh approximately 800 kg and can have a daily feed intake greater than 4.0% body weight in dry matter. At the upper inclusion rate (24 ppm in the feed) on the Canadian premix label (monensin sodium) for dairy cattle, the intake of monensin in some cows could be about 800 mg/head/day, or approximately 1 mg monensin/kg bw/day. In the United States, dairy cattle may be dosed up to 660 mg monensin/head/day, which could result in a dose of approximately 1 mg/kg bw/day in smaller (600 kg) cattle. The upper dose limit for monensin in beef cattle in the United States is 480 mg/head/day, which also approximates to 1 mg/kg bw/day in many cattle at finishing.

Summary of approved uses

The permitted label uses for the monensin CRC and in-feed monensin premix could result in a significant number of cattle (beef and dairy) receiving doses of monensin from 1.0–2.0 mg/kg bw/day. Based on data submitted for evaluation to the 70th meeting of the Committee and submitted for evaluation by the 75th meeting of the Committee, there is a high probability that some cattle treated with monensin in accordance with approved uses will exceed the liver MRL currently adopted by Codex, although the ADI would not be exceeded. Given the safety of monensin in edible tissues, the current adopted Codex MRLs have a potential negative impact on beef trade for major cattle exporting countries. The maximum dose rate used in the world for monensin in cattle is estimated to be 2 mg/kg bw/day.

RESIDUE STUDIES

Two studies were conducted to determine the monensin milk and tissue residues in lactating dairy cattle at zero time withdrawal following the administration of two CRCs and the feeding of premix. In both studies, lactating dairy cows were treated intra-ruminally with two controlled release capsules (32 g monensin in a hexaglycerol distearate matrix in a plastic tube) at day 0. In the first study, previously submitted, cows were fed a medicated ration containing 24 mg monensin/kg feed from day 11 to day 20, and then fed a 36 mg monensin/kg feed for 21 days (Bagg and Dick, 1999). MISSING In the second study (Bagg, 2000) MISSING, submitted for the current evaluation, cows were fed a medicated ration containing 24 mg monensin/kg from day 14 to day 35. After measurement of the monensin release rate from the controlled release capsules, the resulting daily dose ranged from 1537 to 1804 mg monensin per cow (equivalent to 2.4 to 3 mg/kg bw) in the first study and from 778.2 to 1384 mg per cow (equivalent to 1 to 2.4 mg/kg bw) in the second study. At zero time withdrawal, animals were slaughtered and liver and kidney samples were analysed using a validated HPLC method with post-column derivatization (Method AM-AA-CR-R174-AA-791). In the two studies, there were no detectable monensin residues in kidney tissue (<0.025 mg/kg). Monensin residues were detected in 6 of 6 liver samples with two values below the LOQ (detected residues ranged from 0.02 mg/kg to 0.09 mg/kg) collected in the first study, and in one of six livers (25.8 µg/kg) was just above the LOQ (25 µg/kg) in the second study (Table 4.1).

Table 4.1. Relationship between monensin A dosage regimens (2 CRC + premix) and liver residue levels in two studies made available by the sponsor (Bagg, 1999, 2000)

	From Bagg, 1999		From Bagg, 2000	
	Dose (mg/kg bw)	Liver concentration (µg/kg)	Dose (mg/kg bw)	Liver concentration (µg/kg)
	2.7	55.1	2.0	<25
	2.5	20.3*	1.9	25.8
	2.9	69.6	2.0	<25
	2.9	45.8	2.4	<25
	3.0	23.8*	1.0	<25
	2.4	84.5	1.9	<25
Mean	2.7	49.8	1.9	
Min.	2.4	20.3	1.0	
Max.	3.0	84.5	2.4	

NOTES: * = below LOQ (25 µg/kg).

Another study (Terhune, 2007) was completed in 2007. In this study, 9 cattle approximately 18 months of age and weighing from 406 to 537 kg were fed a medicated ration containing 40 mg monensin/kg feed for 24 days with a daily feed consumption around 5 kg per day (estimated to be around 0.4 mg/kg bw). At zero withdrawal time, animals were slaughtered and liver was analysed for monensin using the method: 'Determination of Monensin in Tissues and Eggs (Modified Method 5801654)', a semiquantitative thin-layer chromatography (TLC) autobiographic method with a LOQ of 50 µg/kg. One of the cattle had a monensin liver residue with an estimated concentration of 0.0533 mg/kg (between 0.05 and 0.07 µg/kg). The limit of quantitation for this study was 0.05 mg/kg using a TLC autobiographic method.

Table 4.2. Residues of monensin in dairy cows tissues treated via gelatin capsule at 0.9 mg/kg bw/day as 2 equal doses daily for 7 days

Animal	Time after last dosing	Muscle (µg/kg)	Fat (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)
25	6 h	BLQ	5.24	9.63	1.03
32		BLQ	3.23	9.39	BLQ
39		ND	BLQ	6.42	BLQ
43		BLQ	1.08	10.76	BLQ
16	18 h	ND	BLQ	4.84	BLQ
71		ND	1.41	5.24	BLQ
15		BLQ	BLQ	5.39	BLQ
72		ND	BLQ	6.70	BLQ
3	30	ND	BLQ	2.23	ND
11		ND	BLQ	2.27	ND
75		ND	BLQ	5.43	ND
77		ND	BLQ	2.36	ND

NOTES: BLQ = below limit of quantitation; ND = not detected.

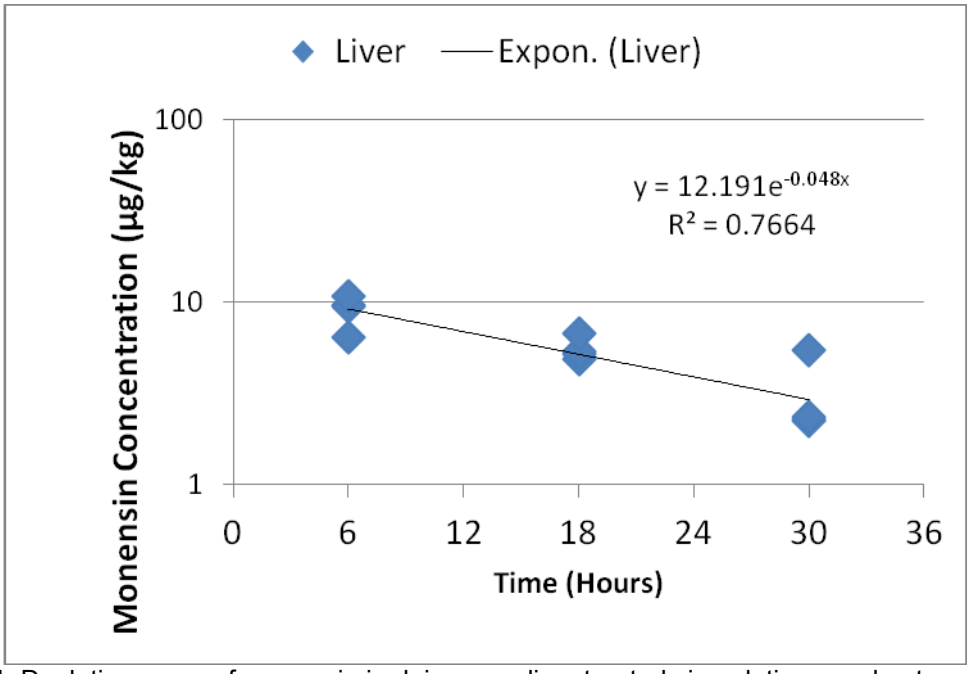


Figure 4.1. Depletion curve of monensin in dairy cows liver treated via gelatin capsule at 0.9 mg/kg bw in 2 equal doses for 7 days

The depletion of monensin was determined in the edible tissues (liver, muscle, kidney and fat) of 12 lactating dairy cows after dosing with monensin at 0.9 mg/kg bw/day for seven consecutive days

(Bassissi and Larvor, 2007). Gelatin capsules containing equal doses were administered at approximately 12-hour intervals. Tissues were collected at 6, 18 and 30 h after the final dosing. Monensin residues were determined using a validated HPLC-MS/MS method with a LOQ of 1 µg/kg (Table 4.2).

The depletion curve obtained in liver is reported in Figure 4.1. The half-life of monensin in liver is about 14 h.

Finally, a study was recently completed to support the registration of a newly designed CRC in Europe (MacDougall and Roberts, 2011). The object of this study was to evaluate the residues of monensin in milk (24 dairy cows) and tissue (10 dairy cows) after 14 days following oral administration of a single monensin CRC. The target dose release for each capsule was 335 mg/day over a 95-day period. Using the daily target dose release and the overall average body weight for cattle in the study, the dose rate was calculated to be 0.53 mg/kg bw/day. Milk samples from each animal were collected twice daily, at 12-hour intervals, immediately prior to treatment (pre-trial) and for 28 consecutive milkings up to 336 h after treatment. Following the morning milking on study day 15, ten animals were slaughtered and tissue samples were collected: liver, kidneys, muscle and fat. All milk samples were initially stored refrigerated until transfer to the analytical laboratory. All tissue samples were stored frozen until analysis. Milk and tissue residue concentrations were evaluated for monensin A using a validated LC-MS/MS method (Charles River Analytical Method No. 1775 Version 1). Due to the nature of the intraruminal CRC, a residue decline phase was not included in this study because the device cannot be removed once administered. Thus, this study provides only one data point, at zero withdrawal. Only tissue sample results are reported.

Table 4.3. Residues of monensin (µg/kg) in the tissues of dairy cows treated via a single CRC device at approximately 0.5 mg/kg bw

Animal	Liver	Kidney	Muscle	Fat
2	2.54	<LOQ	ND	<LOQ
8	23.1	1.29	0.752	5.32
10	18.8	<LOQ	ND	1.07
11	11.2	<LOQ	<LOQ	2.57
13	13.6	<LOQ	ND	<LOQ
17	9.64	<LOQ	ND	1.28
18	26.3	<LOQ	ND	<LOQ
21	13.6	1.45	0.836	2.90
22	22.4	1.37	<LOQ	4.04
24	7.99	<LOQ	ND	<LOQ
Mean	14.9	0.936	<LOQ	2.12
SD	7.53	0.302	0.261	1.55
CV%	50.4	32.2	55.5	73.1

NOTES: ND = not detected.

Samples with no monensin A detected or with a found concentration less than the validated LOD are reported as ND. When a data point is assigned as <LOQ, the LOQ value is assigned for derivation of means and standard deviations. When a data point is assigned as ND the LOD value is assigned for derivation of means and standard deviations. In this study, while the average concentration in liver was below the Codex MRL, several liver samples were determined to be greater than the Codex MRL, and the Upper Tolerance Limit calculated using the arithmetic mean for the 95% confidence limit of the 95th percentile was 36 µg/kg. All other tissue types, including milk, were well below the JECFA MRLs. Results are summarized in Table 4.3.

No new residue data were provided for goat and sheep.

ANALYTICAL METHODS

A method for the determination and confirmation of monensin A and narasin A in cattle liver was validated (MacDougall, 2011). Only the monensin data are relevant to this submission. The laboratory method (CRM 1775) has been formatted according to the ISO 78/2 format. Liver samples are extracted twice with iso-octane/ethyl acetate (90/10). A portion of the combined supernatant is purified by silica solid phase extraction (SPE) before detection and quantification by HPLC with tandem mass spectrometry detection (LC-MS/MS) in the Selected Reaction Monitoring (SRM) mode. Data presented has been quantified for a single transition (693.4>675.6 *m/z*). Data was also collected for confirmatory transitions (693.4>501.6 and 693.4>479.4 *m/z*) but no data was processed or reported for these transitions. Quantification was from a matrix matched calibration line, with 1/x weighting. During the validation work, the European Union MRLs were used as the target concentrations (tissue residue depletion study for submission to the European Medicines Agency, EMA). The Codex MRL (20 µg/kg) is within the validated range for the method (EMA MRL for liver is 30 µg/kg).

System linearity was demonstrated over the range 0.5–100 ng/ml in liver for matrix match calibration standards prepared in extracted control samples for each matrix.

The inter-day assay accuracy and precision was determined for each matrix at their respective ½MRL, MRL and 2MRL (European Union) levels on 3 occasions. The mean intraday assay accuracy and precision for monensin fortified liver ranged from 89.3–103%, with a precision ranging from 2.13 to 9.36%. The inter-day accuracy and precision for liver fortified with 15, 30 and 60 µg/kg monensin was 95.2–101% with a precision no greater than 6.47%.

The specificity of the LC-MS/MS was investigated in liver; the assay was shown to be sufficiently specific. No interference was noted in control samples with a peak area greater than 10% for either test item at the respective LOQ for the matrix. The assay was also shown to be specific against solution standards of penicillin, tylosin, tilmicosin, tetracycline, lasalocid, ceftiofur, ractopamine and ketoprofen.

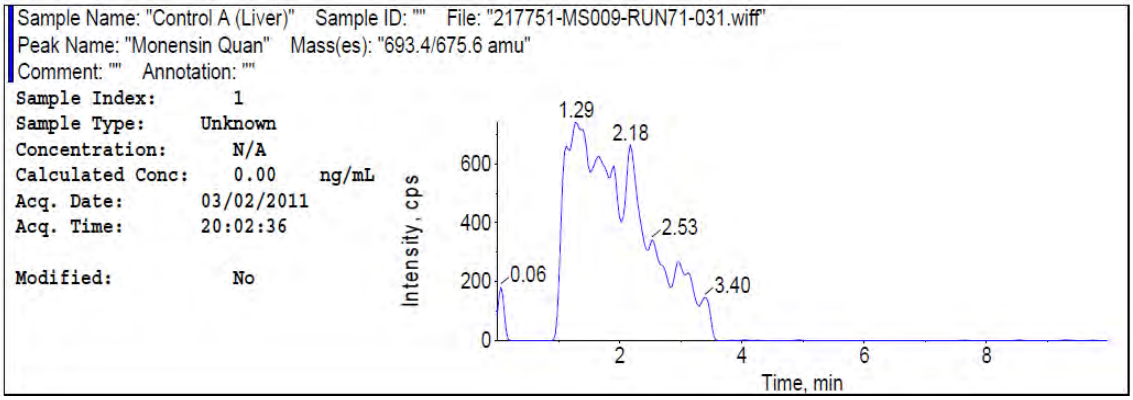


Figure 4.3. Representative ion chromatogram of a control liver extract for monensin (MacDougall, 2011 [217751 Tissue Method Validation, page 147])

Table 4.4. Limits of quantitation and limits of detection are given for Monensin A in liver, kidney, muscle and fat

Matrix	MRL (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)
Liver	30	0.750	0.0823
Kidney	2	0.750	0.0379
Muscle	2	0.750	0.269
Fat	10	1.00	0.0852

The assay LOD was determined by extraction and analysis of 20 aliquots (4 extractions from each of 5 different animals) of the matrix to determine the mean background noise. The LOD was defined as the concentration of each test item equivalent to the mean background noise plus 3 times the

standard deviation. The LOQ for detection of each test item was determined by the extraction and analysis of replicate (n=6) aliquots of control matrix fortified with decreasing concentrations of each test item, and assaying these samples with the standard method. The target intra-day assay accuracy at the LOQ (defined as the mean percentage determined concentration/actual concentration) was 70–110%. The precision at each concentration (defined as the coefficient of variation of the mean determined concentration) was ≤20%. Results are shown in Table 4.4 and Figures 4.4 and 4.5. MRL values referred to are those of the European Union.

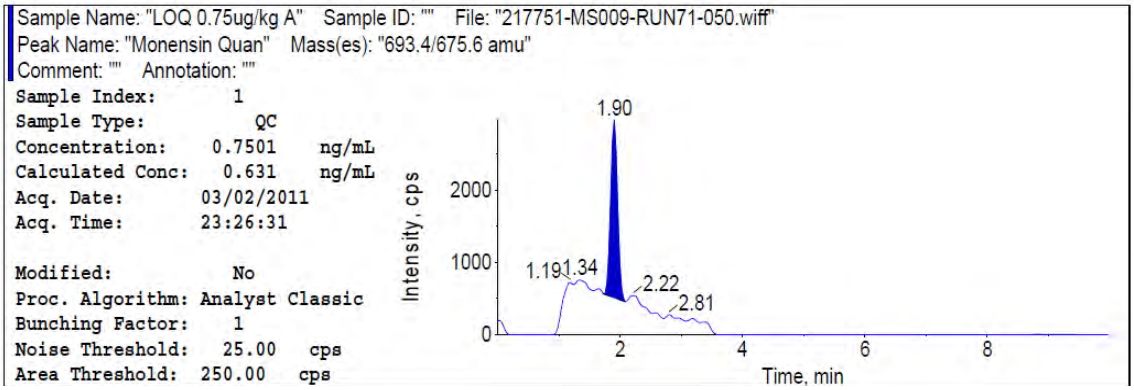


Figure 4.4. Representative ion chromatogram of a liver assay sample fortified with monensin at 0.75 µg/kg (MRL) (MacDougall, 2011 [217751 Tissue Method Validation, page 129])

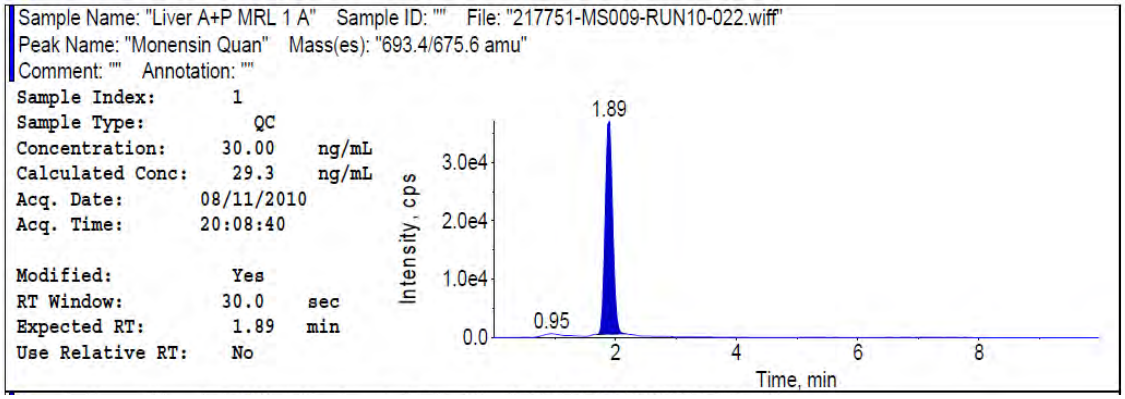


Figure 4.5. Representative ion chromatogram of a liver assay sample fortified with monensin at 30µg/kg (MRL) (MacDougall, 2011 [217751 Tissue Method Validation, page 138])

Stability for monensin A was found acceptable during storage for each tissue at room temperature (i.e. 4 h) and for long (i.e. up to 2 months) frozen storage at -20°C. Its stability after three freeze-thaw cycles was observed for liver. After 1, 2 and 3 freeze-thaw cycles in liver the found concentration difference for monensin at the tested MRL was -14.2, -14.5 and -15.7% respectively compared with freshly extracted samples.

APPRAISAL

The sponsor provided updated information on the different market authorization and practices in cattle using a Controlled Release Capsule (CRC) or medicated feed, or both. The maximum dose rate used in the world for monensin in cattle is estimated by the sponsor to be 2 mg/kg bw/day. The sponsor provided two new study reports and informed the Committee that an additional study is on-going with animals treated with medicated feed, but this study was not available for the present evaluation. The most recent study was based on a new CRC with residue determined by LC/MS-MS.

The sponsor provided residue data based on different modes of treatment and analytical methods. Two studies (Bagg and Dick, 1999, Bagg 2000) combined 2 CRCs with medicated feeding to lactating cows, resulting in 2 dosage regimens (mean exposure: 2.4 mg/kg bw/day and 1.9 mg/kg bw/day). The analytical method was characterized by a LOQ of 25 µg/kg for monensin in liver. In one other study (Bassissi and Larvor, 2007), monensin was administered in gelatin capsule (0.45 mg/kg bw every 12 h for 7 days) to lactating cows. Monensin residues were determined using a validated HPLC-MS/MS method with an LOQ of 1 µg/kg in liver, and a depletion curve obtained.

In a new study (MacDougall and Roberts, 2011), monensin was administered as one CRC to lactating cows, resulting in a mean dosage regimen of 0.53 mg/kg bw/day. The analytical method had a LOQ of 1 µg/kg for monensin in liver. The sponsor hypothesized a linear relationship between dose and monensin concentration in liver. Assuming the same dosage regimen of 1 mg/kg bw/day, Figure 4.6 shows that this assumption is not fully valid. Monensin bio-availability varies according to the administration protocol (CRC alone; 2×CRC+premix; gelatine capsule). Therefore, the concentration of monensin in liver of lactating cows differs between the studies.

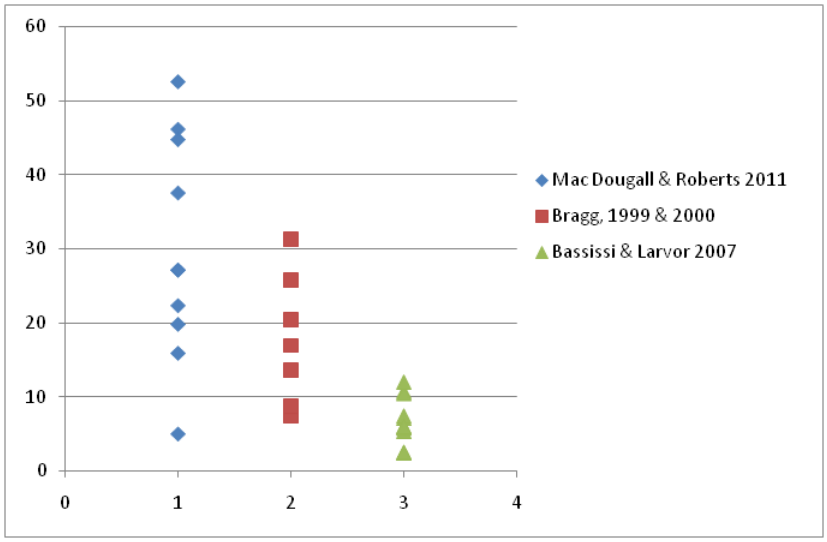


Figure 4.6. Extrapolated monensin concentration in liver (y-axis; µg/kg) for a theoretical dosage regimen of 1 mg/kg bw/day (x-axis) based on studies by MacDougall and Roberts (2011), Bagg (1999, 2000) and Bassissi and Larvor (2007)

The difference in distribution should be related to differences in residue kinetics. The study performed by Bassissi and Larvor (2007) follows an experimental design with a gelatin capsule. The data obtained in the study by MacDougall and Roberts (2011) corresponds to use of a CRC alone in lactating cows. The liver tissue of two animals had monensin concentrations higher than 20 µg/kg. The

tolerance limit (the upper limit of the one-sided 95% confidence interval over the 95th percentile of the linear regression line, the "95/95 tolerance limit") was calculated using the logarithmic transformed monensin concentrations and reached a value of 129 µg/kg at zero withdrawal time. There is uncertainty in the calculation due to the limited number of animals (n=10).

The data obtained in the study (Bagg *et al,* 1999, 2000) are used to describe the concomitant use of monensin CRC and premix. The monensin concentrations in liver were estimated for a maximal dose of 2 mg/kg. The 95/95 tolerance limit was calculated using the logarithmic transformed monensin concentrations, and reached a value of 222 µg/kg at zero withdrawal time. Again, there is uncertainty in the calculation due to the limited number of animals with quantifiable liver concentrations (n=5).

The study of MacDougall and Roberts (2011) employed an approved use of CRC. The monensin concentrations in liver were higher than the MRL of 20 µg/kg in three lactating cows. The 95/95 tolerance limit calculated with logarithmic concentration was 129.6 µg/kg. The uncertainty in the calculation is explained by the limited number of animals (n=10).

The effect of combination of CRC and medicated feed at a maximal dose of monensin 2 mg/kg bw/day on monensin residue in liver is estimated using experimental data (Bagg and Dick, 1999; Bagg, 2000). This scenario is realistic because it is very close to the actual dose administered. However, the dataset is limited (n=5 quantifiable values; 7 values below the LOQ of 25 µg/kg). Consequently, the 95/95 tolerance limit (222 µg/kg) calculated using logarithmic concentrations is far from 100 µg/kg. Using arithmetic concentrations, the 95/95 tolerance limit is calculated to be 115 µg/kg.

MAXIMUM RESIDUE LIMITS

In recommending a revised MRL for monensin for cattle liver, the Committee considered the following factors:

- An ADI of 0–10 µg/kg bw was established by the 70th meeting of the Committee based on a chronic toxicological end-point. This ADI is equivalent to up to 600 µg monensin for a 60 kg person.
- Monensin A is a suitable marker residue in liver.
- Monensin A is extensively metabolized and represents conservatively 5% of total residues in tissues.
- Different oral formulations of monensin and intra-ruminal CRCs are approved for use in cattle or lactating cows. Concomitant administration of these formulations and intraruminal controlled release capsules would lead to a maximum daily dose regimen of up to 2 mg/kg bw, according to the sponsor.
- At zero withdrawal time, one GLP study based on the administration of one CRC to lactating cows showed that the existing MRL for liver, originally set at 20 µg/kg, was exceeded. The 95/95 tolerance limit of monensin A in cattle liver was calculated as slightly above 100 µg/kg. This value is explained partly by the uncertainty associated with the low number of animals (10) slaughtered at zero withdrawal time.
- Using the data issued from the over-dosage studies conducted with a combination of two CRCs and medicated feed, liver concentrations higher than 25 µg/kg were reported for dose rates higher than 2 mg/kg bw/day. Under the assumption of dose linearity, for a maximum daily dose of 2 mg/kg bw the 95/95 tolerance limit leads to a value significantly higher than 100 µg/kg. This value is explained by the uncertainty associated with the low number of reported values and lack of information on the residue concentration below the method LOQ (25 µg/kg).
- For goat and sheep, no additional information was provided by the sponsor. Without any additional data, the Committee was unable to revise its recommendation on liver MRLs for goat and sheep.
- A validated HPLC-MS/MS complete method with adequate performance parameters and method validation was provided and was considered suitable for routine monitoring of monensin A as marker residue. On the basis of the residue study performed with the CRC administered alone to lactating cows, the Committee recommended a revision for MRL for cattle liver to 100 µg/kg, determined as monensin A.
- Using the model diet, these MRLs would result in an intake of 481 µg/day per person, which represents 80% of the upper bound of the ADI.
- The combined use of the controlled release capsule and premix in cattle at the highest dosage reported by the sponsor will be likely to result in a residue in excess in liver, over the MRL of 100 µg/kg.

Table 4.5. Maximum daily intake calculated for the standard food basket for the recommended MRLs in bovine tissues

Tissue	Consumption factor (kg)	Codex MRL (µg/kg)	Ratio (MR:TRR)	Quantity ingested (µg total residue)
Muscle	0.300	10	0.05	60
Fat	0.050	100	0.05	100
Liver	0.100	100	0.05	200
Kidney	0.050	10	0.05	10
Milk	1.500	2	0.027	111
			TDMI (µg/person)	481
			ADI (µg/person)	600
			% of ADI	80

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Terhune, T. 2007. Non-clinical laboratory study: Residue depletion in beef cattle following oral administration of monensin, ractopamine, tylosin and melengestrol acetate (heifers only) for 24 days. Unpublished Study Number HMS 041004. Conducted for Elanco Animal Health by HMS Veterinary Development, Tulare, CA, USA. [Includes Final Study Report Amendment #1.]

Monepantel

First draft prepared by
Joe Boison, Saskatoon, Canada
and
Pascal Sanders, Fougères, France

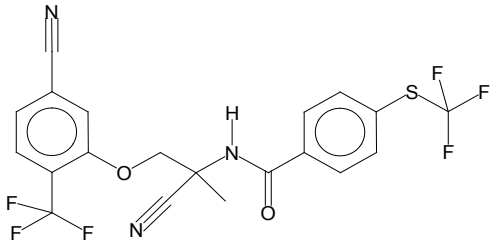
IDENTITY

IUPAC Name: N-[(1S)-1-Cyano-2-(5-cyano-2-trifluoromethyl-phenoxy)-1-methyl-ethyl]-4--trifluoromethylsulfanyl-benzamide

Synonyms: N-[2-(5-cyano-2-trifluormethyl-phenyloxy)-1-(S)-1-cyano-1-methyl-ethyl]-4-trifluoromethylthio-benzoic amide

Chemical Abstracts Service Number: 887148-69-8

Structural formula:



Molecular formula: $C_{20}H_{13}F_6N_3O_2S$

Molecular weight: 473.4

Other information on identity and properties

Pure active ingredient: AHC-2102225 (N-[2-(5-cyano-2-trifluormethyl-phenyloxy)-1-(S)-1-cyano-1-methyl-ethyl]-4-trifluoromethylthio-benzoic amide), the active S-enantiomer

Appearance: White powder

Melting point: 125°C (polymorphic form A;, 142–149°C (polymorphic form B)

Solubility in water: 0.1 mg/L at 20°C

Solubility in organic solvents: dichloromethane: 175 g/L; ethanol: 60.7 g/L; n-octanol: 7.3 g/L; propylene glycol: 6.9 g/L; polyethylene glycol: 156.1 g/L

pH: 6.2–6.3 (suspension in water)

Partition coefficient: Octanol/water partition coefficient: log P_{ow} = 3.0 (shake flask method, pH 7, at 20°C)

Storage: At room temperature, protect from light

Chirality: Monepantel has one chiral centre

Optical Density: Optical rotation $[\alpha]_{580nm}$ -32° (methanol)

Purity: AHC-2155367 = N-[2-(5-cyano-2-trifluormethyl-phenyloxy)-1-(S)-1-cyano-1-methylethyl]-4-chloro-benzoic amide, residual solvents, etc., each specified at <0.5%

RESIDUES IN FOOD AND THEIR EVALUATION

Conditions of use

Monepantel is an anthelmintic of the amino-acetonitrile derivative class, indicated for the treatment and control of gastrointestinal roundworms (nematodes) in sheep. It is marketed under the trade name Zolvix® and licensed for use in Australia, New Zealand, Switzerland, South Africa, Uruguay and Argentina.

Dosage

The recommended dose is as a single oral drench of 2.5 mg/kg bw and the maximum dose used is 3.75 mg/kg bw. The label has a warning that the product should not be administered to female sheep which are producing or may in the future produce milk or milk products for human consumption.

PHARMACOKINETICS AND METABOLISM

Absorption, distribution, metabolism and excretion

Sheep

A non-GLP-compliant pilot study investigating the absorption, distribution, metabolism and excretion (ADME) of ^{14}C-monepantel, following oral dosing, in two sheep was conducted (Jung, 2006). ^{14}C-monepantel diluted with unlabelled drug was dissolved in a formulation similar to the proposed final formulation. Blood, urine and faeces were collected for 12 days post-treatment, at which time the animals were sacrificed for the collection of tissues. Total radioactive residue (TRR) in collected samples was measured and the metabolic profiles determined by LC-MS and HPLC/LSC (liquid scintillation counter) in selected samples, comparing with available authentic reference compounds. Due to the non-homogeneity of the test formulation, the doses administered were less than anticipated, and results could only be estimated. Consequently, accurate figures for excretion and balance were not possible. A large fraction (about 90%) of the dose was excreted via the urine and faeces, although the latter contained a significant proportion of unabsorbed drug over the first 1–2 days. Between animals there was variability in the ratio of faecal versus urinary excretion, ranging from 2:1 to 3:1 during the last few days before sacrifice. Figure 5.1 shows the cumulative excretion profile of radioactivity after a single oral administration of ^{14}C-monepantel to one of the sheep. The remainder of the total radio-activity (17–27%) was distributed in fat (11–21%) and muscle (4%). The distribution of radioactivity in edible tissues is summarized in Table 5.1.

Radioactivity in blood peaked between 8 and 24 h after administration, and declined slowly thereafter. Radioactivity was neither exclusively bound in the plasma fraction, nor in the red cell fraction. Liver and fat were the tissues with the highest radioactivity. Muscle and kidney were relatively low in residues, and the variability observed for muscle residues may reflect the variability in fat content, as drug residues are lipophilic in nature. Various extraction experiments were conducted to determine the recovery of incurred radioactivity from samples. In general, simple extraction with organic solvents yielded 90% or better recovery, except for liver, where only about 70% was recovered following high-speed homogenization with acetonitrile (or other tested solvents).

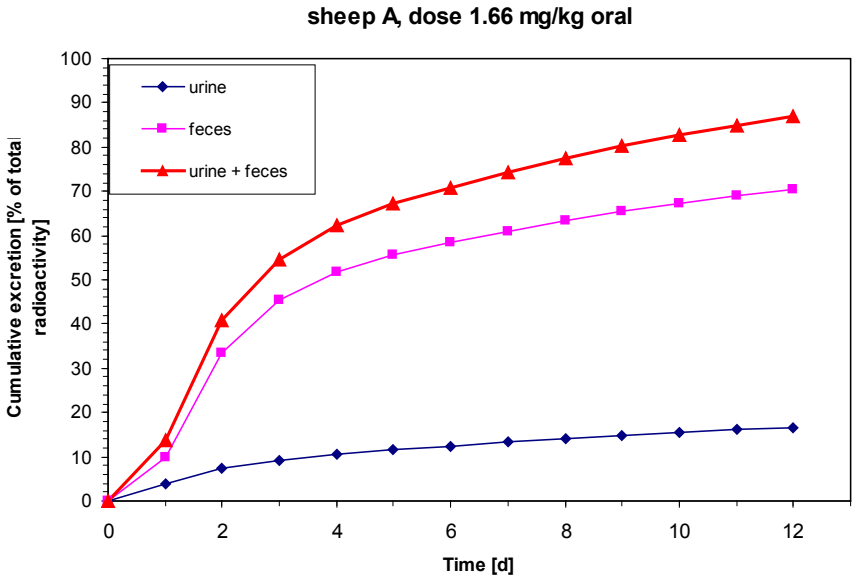


Figure 5.1. Cumulative excretion of radioactivity after oral administration of 14C-monepantel at 1.66 mg/kg bw to sheep

Table 5.1. Distribution of radioactivity in edible tissues of sheep (pilot study)

Tissue	Monepantel equivalents (µg/kg) 12 days after treatment	
	Sheep A (dose ~1.7 mg/kg bw)	Sheep B (dose ~4.6 mg/kg bw)
Fat (subcutaneous)	1887	2665
Fat (peritoneal)	2250	3402
Liver	884	1932
Kidney	228	392
Muscle (shoulder)	330	979
Muscle (thigh)	169	342
Skin	204	324
Blood	11	28
Plasma	10	31
Wool (back)	23	63

Metabolite profiling indicated that in tissue, blood, plasma and faeces, the sulphone metabolite is predominant, together with minor amounts of parent drug. For blood and faeces, the initial samples had a proportionately higher amount of the parent drug, especially in faeces collected within two days of treatment. Trace amounts of sulphoxide metabolite were observed in blood at 8 h post treatment, and also in faeces up to two days post-treatment.

Faeces contained two additional metabolites: the phenol M4, and the hydroxylated sulphone M3. Urine contained no parent compound or oxidized (unconjugated) metabolites of the parent. Two metabolites were observed; the minor one was M4 and the major one was its sulphate conjugate M5. The latter metabolite was confirmed by hydrolysis with sulphatase to M4. The structures of monepantel and identified metabolites are shown in Figure 5.2.

A GLP-compliant study investigating the ADME and residue depletion of ^{14}C-monepantel, using test material labelled at either of two rings (on the cyano group, referred to as label 2, or on the amide group, referred to as label 3) of the parent molecule, was reviewed (Jung *et al*., 2007). The radioactive substances were dissolved in formulation TG 1778/30, and 17 male and 17 female Suffolk sheep were orally dosed at 5.0 mg/kg bw with either label 2, label 3 or an equimolar mixture of each labelled

substance (labels 2+3). Blood, excreta, wool and edible tissues (fat, muscle, kidney and liver) were collected 2, 7, 14, 21, 28 and 35 days post-dose and analysed for TRR. Tissue residues were extracted and the analytes quantified using the validated HPLC/UV (LOQ = 0.05 µg/kg) method (Karadzovska, 2007a). Blood and plasma were extracted and analysed by the validated method for blood for monepantel and monepantel sulphone (Karadzovska, 2007b).

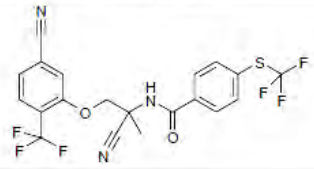
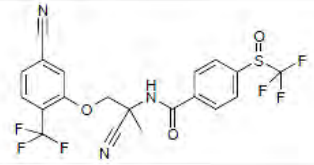
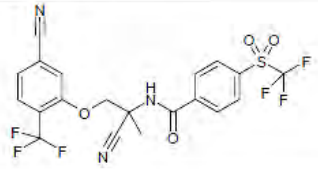
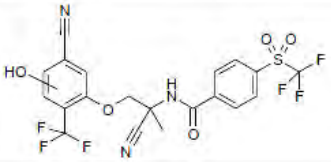
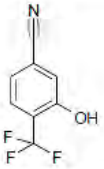
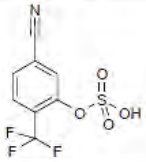
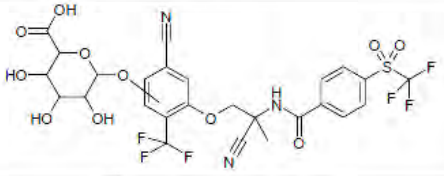
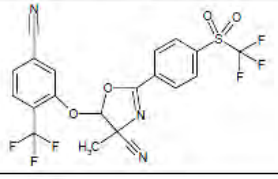
Metabolite ID	Code ID	Description/other names	Structure
Parent (monepantel)	AHC-2102225	NG-96 is the racemate	
M1		Sulfoxide of parent	
M2	AHC-2144670	Monepantel sulfone NG-236 is the racemate	
M3		Hydroxylated M2	
M4	AHC-2166636	Phenol	
M5	AHC-2166637	Sulfate of phenol	
M6		Glucuronide of M3	
G32	AHC-2197876	M32	

Figure 5.2. Identified metabolites of monepantel

The cumulative excretion is summarized in Table 5.2. The data show that radioactivity is predominantly excreted through the faeces, with a significant contribution from urinary elimination. Faecal excretion is high in the first 3 days (30%), but subsequently the rate declines, with about 2–3 weeks required for 90% elimination. Blood and plasma profiles, obtained from 4 sheep dosed with the

label 2 substance, are shown in Table 5.3. Data show slow elimination from the systemic circulation, and radioactivity is distributed approximately equally between the cellular and plasma compartments.

Table 5.2. Mean cumulative excretion of radioactivity from sheep as % of dose

Label	No. of sheep	Days	Urine (%)	Faeces (%)	Cage Wash (%)	Total (%)
2	2	0 – 14	30.8 ± 1.3	52.9 ± 5.0	1.8 ± 0.3	85.4 ± 6.0
3	2	0 – 14	28.8 ± 0.8	60.6 ± 0.7	2.2 ± 1.1	93.3 ± 1.5
		0 – 14	29.2 ± 5.8	52.8 ± 4.1		
2 + 3	4	0 – 21	30.7 ± 6.0	55.3 ± 3.4	4.2 ± 2.5	90.2 ± 5.4

Table 5.3. Mean sheep blood and plasma radioactivity profiles expressed as monepantel equivalents

Day	Blood (mean ± SD) (µg/kg equivalents)	Plasma (mean ± SD) (µg/kg equivalents)
1	254 ± 27	300 26
2	145 ± 26	176 ± 21
4	77 ± 21	91 ± 23
7	53 ± 21	63 ± 23
14	31 ± 17	36 ± 20
21	19 ± 14	21 ± 15
28	9 ± 9	12 ± 9

Residues in tissues and other matrices are shown in Table 5.4. The position of the label did not affect the excretion or distribution of residues. The highest residues in edible tissues were found in the fat, with slightly higher residues in rendered pure fat compared with composite fat tissue, followed by liver, kidney and then muscle. The approximate TRR proportions were 100 (fat):50 (liver):20 (kidney):10 (muscle):1 (blood):1 (plasma), respectively.

Table 5.4. Depletion of total radioactive residues (TRR) from sheep tissues

	Mean TRR ± SD in mg equivalents/kg							
Label position	2	2	2	3	2	2+3	2	2
Days post-dose	2	7	14	14	21	21	28	35
Bile	4.6 ±1.6	1.1 ±0.52	0.34 ±0.24	0.33 ±0.17	0.13 ±0.10	0.08 ±0.10	0.19 ±0.23	0.08 ±0.09
Blood	0.14 ±0.20	0.04 ±0.10	0.02 ±0.10	0.02 ±0.10	0.01 ±0.01	0.01 ±0.00	0.01 ±0.01	0.01 ±0.00
Plasma	0.16 ±0.04	0.05 ±0.01	0.03 ±0.01	0.02 ±0.01	0.01 ±0.01	0.01 ±0.00	0.01 ±0.01	0.01 ±0.00
Fat tissue	15.5 ±4.0	5.8 ±2.9	2.2 ±1.2	1.7 ±0.83	1.1 ±0.50	0.74 ±0.52	1.1 ±0.62	0.46 ±0.26
Pure fat	19.3 ±5.2	7.3 ±2.3	2.9 ±1.4	2.1 ±0.90	1.3 ±0.57	0.99 ±0.52	1.3 ±0.67	0.55 ±0.28
Liver	6.7 ±0.23	2.7 ±0.75	1.5 ±0.71	1.1 ±0.47	0.77 ±0.37	0.50 ±0.33	0.71 ±0.55	0.33 ±0.25
Kidney	2.4 ±0.15	0.81 ±0.26	0.38 ±0.24	0.32 ±0.16	0.16 ±0.10	0.12 ±0.08	0.18 ±0.1	0.06 ±. 06
Muscle	1.50 ±0.34	0.45 ±0.23	0.22 ±0.15	0.14 ±0.08	0.11 ±0.06	0.06 ±0.05	0.09 ±0.06	0.03 ±0.03

The distribution of non-radiolabelled monepantel and its metabolite, monepantel sulphone in tissue, is summarized in Table 5.5. The approximate proportions of monepantel sulphone in the tissues were 10 (fat):5 (liver):2 (kidney):1 (muscle).

Table 5.5. Depletion of monepantel and its metabolite monepantel sulphone from sheep tissues

Day	Label position	Fat Tissue	Pure Fat	Liver	Kidney	Muscle
Monepantel [mean concentration ± SD (mg/kg)]						
2	2	3.5 ± 1.4	5.1 ± 2.0	0.39 ± 0.12	0.14 ± 0.05	0.28 ± 0.08
7	2	0.61 ± 0.41	0.92 ± 0.61	All <0.05	All <0.05	0.81 + 3 at <0.05
14	2, 3	0.09 ± 0.02 + 3 at <0.05	0.13 ± 0.04 + 3 at<0.05	All <0.05	All <0.05	All <0.05
21	2, 2 + 3	All <0.05	All <0.05	All <0.05	All <0.05	All <0.05
Monepantel sulphone [mean concentration ± SD (mg/kg)]						
2	2	10.2 ± 2.1	13.4 ± 3.1	5.2 ± 0.13	1.5 ± 0.23	1.4 ± 0.32
7	2	4.2 ± 2.0	5.7 ± 2.5	1.9 ± 0.57	0.60 ± 0.26	0.47 ± 0.26
14	2, 3	1.6 ± 1.0	2.2 ± 1.2	0.87 ± 0.49	0.30 ± 0.08 + 2 at <0.05	0.20 ± 0.10 + 2 at <0.05
21	2, 2 + 3	0.60 ± 0.56	0.76 ± 0.67	0.34 ± 0.30	0.13 ± 0.07 + 3 at <0.05	0.15 ± 0.06 + 5 at <0.05
28	2	1.1 ± 0.47 + 1 at <0.05	1.5 ± 0.73 + 1 at <0.05	0.550 ± 0.32 + 1 at <0.05	0.18 ± 0.10 + 1 at <0.05	0.15 ± 0.07 + 2 at <0.05
35	2	0.60 ± 0.02 + 2 at <0.05	0.70 ± 0.09 + 2 at <0.05	0.26 ± 0.04 + 2 at <0.05	0.10 ± 0.01 + 2 at <0.05	0.06 ± 0.01 + 2 at <0.05

Metabolite identification and profiling of residues in sheep samples

Selected samples of edible tissues (except pure fat), blood, wool and excreta were extracted and profiled by HPLC with LSC detection. If a simple extraction failed to recover a large proportion of TRR, then further extractions with harsher conditions were attempted.

Some samples were extracted and profiled after prolonged storage in the freezer and compared with the initially obtained results; there were no significant changes in the profiles, indicating acceptable stability (except for urine). HPLC profiling of selected blood samples on a chiral column indicated that there was no racemization of parent or sulphone. Attempts were made to profile bile and wool, but no conclusions could be drawn because of poor extractability, low levels or complex profiles. Monepantel was not detected, but the sulphone and the M6 may have been present in bile. The proposed metabolic pathway for monepantel in sheep is shown in Figure 5.3.

The chromatographic and spectral data support the proposed metabolites and pathways. The information obtained formed the basis for selection of the marker residue and the regulatory analytical method. The distribution of metabolites in edible sheep tissues was remarkably comparable to those obtained during non-radiolabelled drug analysis and metabolite profiling studies. Little or no bound residues were observed in edible tissues. There are two main metabolic pathways:

- One route involves oxidation of the parent to the transient sulphoxide M1, with rapid oxidation to the sulphone M2. There is a further slow oxidation to M3; the site of the hydroxylation was not elucidated but was thought to be on the phenolic ring.
- The other route involves cleavage to yield the phenol M4, together with its sulphate conjugate M5. From the corresponding benzamide portion, an alcohol and a peptide hydrolysis product, an acid that is formed and eliminated via the urine.

Elimination is predominantly via faeces, with significant contribution from urine. Bile excretion contributes to total faecal elimination. A tiny fraction of the drug is excreted with wool.

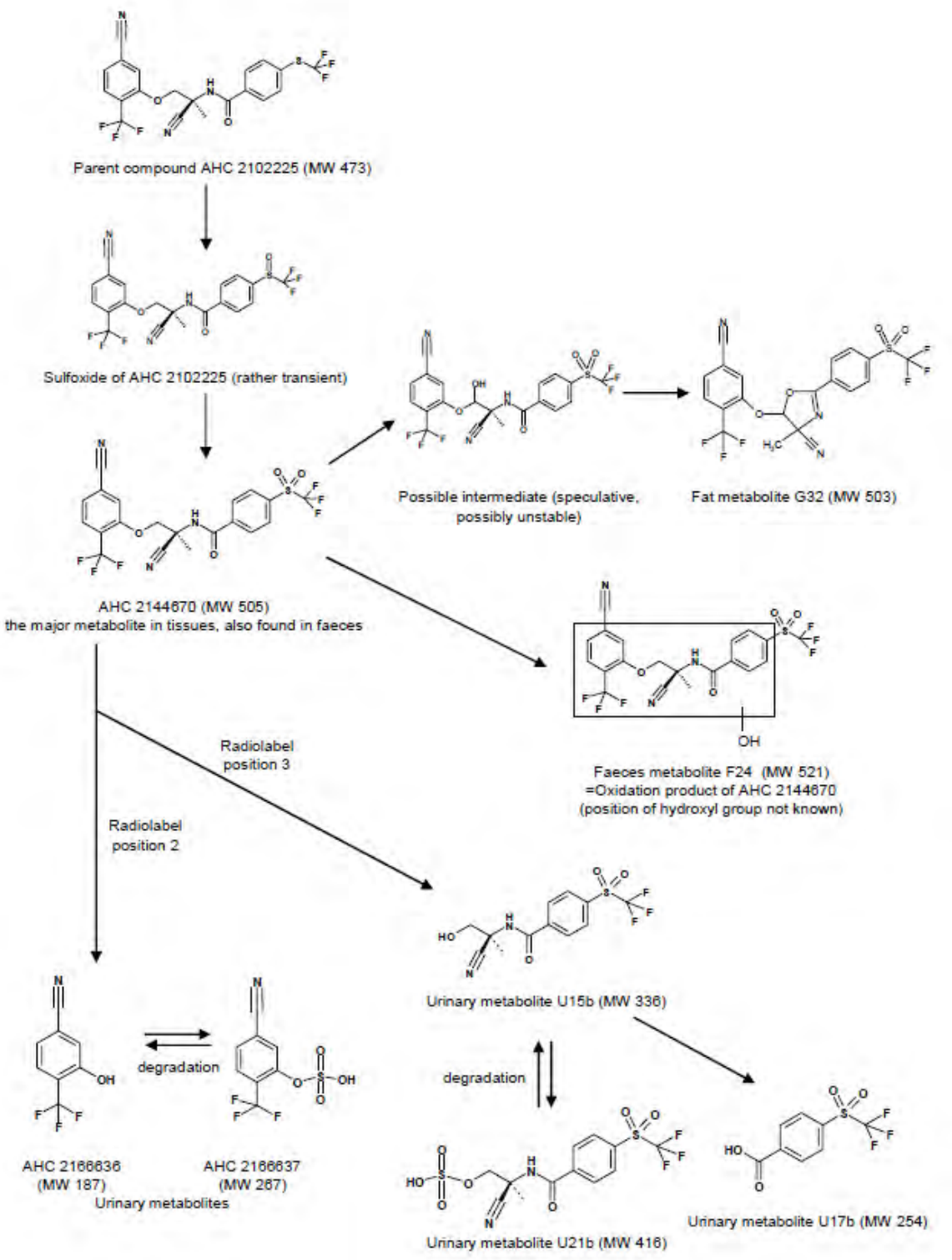


Figure 3. Proposed metabolic pathway of monepantel in sheep

Radioactive residues in fat tissue (most samples) were readily extractable with hexane (>90%) and the metabolites were identified. The sulphone was a dominant metabolite, with monepantel being a minor contributor at early times. A cyclized metabolite (G32), appeared at day 7, and was still observed at 35 days. Its distribution, as a percent of TRR, was variable between animals and increased with time to about one-third (mean value) of TRR at day 35. However, the highest individual residues (400 µg/kg) occurred at 7 to 21 days post-treatment.

Radioactive residues in liver (selected representative samples only) were readily extractable with acetonitrile at room temperature (70%) and more vigorous conditions (polar solvents, reflux, extreme pH) could extract another 16%, suggesting a very small amount of bound residues. Monepantel sulphone was the major metabolite, together with minor amounts of parent at day 2, plus two or three minor metabolites that were less than 10% of TRR. The metabolite G32 was not detected.

Kidney samples (selected representative samples only) also were readily extractable with acetonitrile at room temperature (80%). The pattern of metabolites was very similar to that of liver, with some additional very minor metabolites, one of which is tentatively the sulphoxide. Again, G32 was not detected.

Radioactivity in muscle samples (selected representative samples only) was readily extractable with acetonitrile at room temperature (90%). The sulphone was the major metabolite, with minor amounts of parent only at day 2. Minor amounts of G32 (<14 µg/kg) were observed in some samples at day 14 and 21 (only these samples were profiled), contributing between 0 and 27% to individual TRR.

The higher percentages of G32 were found in those animals with high contributions of G32 to fat TRR. It is reasonable to assume that G32 in muscle tissues arises from intramuscular fat, which is naturally present. G32 was not detected at day 2. Faeces metabolites were readily extractable with acetonitrile/water, and were dominated by (unabsorbed) parent during the first few days, and then by the sulphone and M3. Some minor polar metabolites, such as the phenol, were also observed.

A complex pattern was observed in urine, which contained numerous polar metabolites resulting from the cleavage of the ether linkage in monepantel, and, in some cases, followed by conjugation. The major label-2 urinary metabolites were identified as the phenol and its sulphate, the latter being unstable and degrading to the former. The major label-3 urinary metabolites were the alcohol formed after cleavage of the phenylether bond (U15b) plus its corresponding sulphate (U21b) and the carboxylic acid formed after cleavage of the peptide bond (U17b). G32 was not detected in excreta or in blood.

Pharmacokinetics

A pharmacokinetic study of monepantel and its main metabolite, monepantel sulphone, was performed in 36 sheep, 18 castrated males and 18 females aged 6–8 months (Karadzovska, 2007c). The animals were allocated to five treatment groups on the basis of sex and body weight. In the first group of 6 animals, monepantel was administered intravenously at a dose of 1 mg/kg bw. In the second group of 6 animals, monepantel sulphone was administered intravenously at a dose of 1 mg/kg bw. The three remaining groups of 8 animals received monepantel via oral route at a dose of 1, 3 or 10 mg/kg bw. Blood specimens were collected from the animals in the groups treated intravenously at pre-defined time points: pre-treatment, 2, 5, 10, 30 minutes, 1, 2, 4, 8, 12, 24, 36, 48, 72, 96 h and 7, 10, 14, 21, 28 days post-treatment. Blood specimens were collected from the animals in the groups treated orally at pre-defined time points: pre-treatment, 30 minutes, 1, 2, 4, 8, 12, 24, 36, 48, 72, 96 h, and 7, 10, 14, 21, 28, 35 days post-treatment. Specimens were analysed for monepantel and its metabolite, monepantel sulphone (LOQ = 3 ng/ml). Faecal specimens were collected from groups 1, 2 and 4 over two 8-hour periods to estimate faecal clearance. The calculated pharmacokinetic parameters after i.v. or oral administration of monepantel are summarized in Table 5.6.

Table 5.6. Pharmacokinetic parameters of monepantel after i.v. and oral administration

Route	Intravenous	Oral	Oral	Oral
Dose (mg/kg bw)	1	1	3	10

Parameter (Unit)				
$AUC_{0\text{-}7d}$ (ng-h/ml)	676.1 ± 89.3	220.4 ± 78	703.1 ± 234.4	1967.2 ± 472.4
T_{max} (h)		2-8	4-24	4-6
C_{max} (ng/ml)		7 ± 1.9	18.9 ± 6.9	130.4 ± 110.2
MRT (h)	5.3 ± 2	24.0 ± 5.7	30.0 ± 3.2	22.3 ± 4.9
CL (L/(kg-h))	1.5 ± 0.2			
Vss (L/kg)	7.68 ± 2.34			

After i.v. administration of 1 mg/kg bw of monepantel, the blood monepantel concentration fell rapidly and the last quantifiable blood level was detected at 48 h. Total blood clearance was high (1.49 L/kg/h) and it was not possible to determine the half-life of the parent drug. Peak blood concentration of monepantel sulphone was achieved approximately 2 h after i.v. administration of monepantel, and monepantel sulphone concentrations remained above the LOQ for more than 7 days, depending on the animal. The calculated pharmacokinetic parameters after i.v. and oral administration of monepantel and i.v. administration of monepantel sulphone are summarized in Table 5.7. After i.v. administration of 1 mg/kg bw of monepantel sulphone, monepantel sulphone concentration remained above the LOQ for more than 4 days. Total blood clearance was smaller than for the parent compound (0.28 L/kg/h) and the terminal half-life was approximately 4.5 h. The volume of distribution at steady state of monepantel sulphone was 31.2 L/kg.

Bio-availability of monepantel after oral administration of 1 mg/kg bw was approximately 31% ($Cl_{90\%}$ = 23–44%). Calculated bio-availability of monepantel sulphone obtained after oral administration of 1 mg/kg bw of monepantel was 94% ($Cl_{90\%}$ = 71–124%). It is not a true bio-availability because the animals are different, but it shows that approximately the same amount of monepantel sulphone is formed whether monepantel is given orally or intravenously, hence demonstrating that the oral route has an excellent bio-availability of amounts of monepantel sulphone generated after administration of monepantel. The difference in oral bio-availability of monepantel and monepantel sulphone can be explained by a complete first-pass effect and a total absorption of the oral dose of monepantel.

Dose linearity of the blood concentration of monepantel after oral administration of monepantel appears to hold for oral administration of 1–10 mg/kg bw but is only seen for the dose of 3 mg/kg bw for blood concentration of monepantel sulphone (Table 5.7). Dose corrected AUC, terminal half-life and MRT are significantly lower at 10 mg/kg bw than at 1 mg/kg bw.

A small fraction of monepantel (4%) is excreted unchanged in faeces and the great majority is converted to monepantel sulphone (94%). This confirms that the conversion of monepantel into monepantel sulphone is the most important metabolic pathway. When monepantel sulphone was administered, 27% was excreted in faeces.

Table 5.7. Pharmacokinetic parameters of monepantel sulphone after i.v. and oral administration of monepantel and i.v. administration of monepantel sulphone

Route	Intravenous	Intravenous	Oral	Oral	Oral
Compound	Monepantel	Monepantel sulphone	Monepantel	Monepantel	Monepantel
Dose	1 mg/kg bw	1 mg/kg bw	1 mg/kg bw	3 mg/kg bw	10 mg/kg bw
Parameter (Unit)					
$AUC_{0\text{-}7d}$ (ng-h/ml)	2344 ± 290	2705 ± 631	2 442 ± 433	7 207 ± 1 230	1519 ± 1 251
$AUC_{0\text{-}\infty}$ (ng-h/ml)	3651 ± 721	3701 ± 1056	3 564 ± 1 419	11 571 ± 3 600	19 200 ± 1 937
T_{max} (h)			24	24	24
C_{max} (ng/ml)			30.3 ± 4.6	31.8 ± 5.4	29.4 ± 12.2
MRT (h)	127.47 ± 43.14	119.79 ± 46.58	139.15 ± 43.58	170.3 ± 47.15	104.47 ± 32.18
CL (L/(kg-h))		0.292 ± 0.096			
Vss (L/kg)		32.13 ± 8.27			

TISSUE RESIDUE DEPLETION STUDIES

Residue studies with radiolabelled monepantel

A pivotal ADME study was conducted in sheep, with radiolabelled monepantel to demonstrate the depletion of residues in edible tissues with a non-final formulation, at a dose rate (5 mg/kg bw) higher than the maximum recommended rate of 3.75 mg/kg bw.

The collected tissue and blood samples were analysed by HPLC with UV detection for monepantel and monepantel sulphone (Karadzovska, 2007b). Tissues were extracted once with acetonitrile and the analytes quantified using the validated analytical method; results were summarized earlier, in Table 5.5. Blood and plasma were analysed by the method validated for blood. Blood levels were consistent with data observed in pharmacokinetic studies and plasma results confirmed the blood:plasma distribution ratio of about 1, for both parent drug and monepantel sulphone. The ratios of sulphone were 200 (fat):100 (liver):30 (kidney):20 (muscle):1 (blood):1 (plasma).

The ratio of the concentration of monepantel sulphone (marker residue) to total radioactive residue (expressed as equivalent monepantel concentration) for each tissue was calculated using all valid data provided. Mean ratios of marker residue:total residues of 1 for muscle and of 0.66 for liver, kidney and fat were determined.

Residue depletion studies with unlabelled monepantel

Three GLP-compliant residue depletion studies using unlabelled monepantel were reviewed. The final commercial formulation at the proposed maximum dose rate of 3.75 mg/kg bw was used, with a single administration individually adjusted to the individual animal body weight. In all 3 studies, equal numbers of females and castrated males were used, and sheep were maintained on green pasture. Renal fat, subcutaneous back fat (if present), liver, kidney and muscle were collected and analysed for monepantel sulphone only, using a validated analytical method (Karadzovska, 2007a, b). The additional SPE clean-up was used to reduce the LOQ to 10 µg/kg, and consequently increase the number of quantifiable values for statistical purposes.

Residue depletion in Suffolk lambs after single dose administration

Thirty two Suffolk lambs, 3–4 months old were used (Karadzovska, 2007d). The mean dose received was 3.8 mg/kg bw. A group of four animals served as controls. Groups of eight were sacrificed at 7, 18, 29 and 40 days after treatment. Mean residues are shown in Table 5.8.

Table 5.8. Monepantel sulphone residues in edible tissues of Suffolk lambs after a single oral administration of monepantel

Day	Mean residue concentration ± SD (µg/kg)				
	Renal fat	Subcutaneous fat	Liver	Kidney	Muscle
7	3256 ± 1106	2 417 ± 1 153	1 757 ± 516	591 ± 201	222 ± 114
18	490 ± 326	538 ± 281	212 ± 141	71 ± 52	43 ± 17 + 3 <10 µg/kg
29	115 ± 67 + 1 <10 µg/kg	114 ± 38	91 ± 55 + 1 <10 µg/kg	21 ± 9 + 2 <10 µg/kg	15 ± 3 + 5 <10 µg/kg
40	109 ± 73 + 2 <10 µg/kg	114 ± 78	59 ± 43 + 2 <10 µg/kg	18 ± 9 + 4 <10 µg/kg	12 + 7 <10 µg/kg

Residue depletion in Suffolk lambs after repeated administration

A GLP-compliant repeat dosing study (Smal, 2007) with 5-month-old Suffolk lambs, 30–39 kg, was conducted with equal numbers of females and castrated males, maintained on green pasture. The animals were dosed every 21 days at a rate of 3.75 mg/kg bw, with the dose adjusted each time to the new body weight. Up to 4 doses were administered and the mean dose rate received was 3.7 to 3.8 mg/kg bw. Between the 1st and 2nd doses the animals gained about 25% in weight, but then lost weight (about 3%) between the 2nd and 3rd treatments due to poor pasture growth. Afterwards they gained about 12% in weight. Groups of 6 animals were sacrificed at 21 days after the 2nd and 3rd doses, and at 14 and 21 days after the 4th dose. Samples were analysed for monepantel and monepantel sulphone only to the LOQs of 50 µg/kg, using the validated analytical procedure. There were no quantifiable residues of monepantel, and mean residues of monepantel sulphone were compared with the single-dose Suffolk lamb study. The results are shown in Table 5.9.

Table 5.9. Comparison of residues of monepantel sulphone after repeat dosing of monepantel

Study	Doses/sacrifice	Renal fat	Liver	Kidney	Muscle
Y06/93	2/21 days	133	108	<50	<50
	3/21 days	<50	<50	<50	<50
	4/14 days	395	305	80	<50
	4/21 days	328	227	66	<50
Y07/21	1/18 days	490	212	71	<50

NOTES: Values the mean (n = 6) of residues of monepantel sulphone (µg/kg). For calculation of means, residues <LOQ were replaced by half LOQ.

Residue depletion in cross-bred lambs

In another study, 47 second-cross-bred lambs (Merino × Dorset), 3–4 months old, were used (Karadzovska, 2007e; Strehlau, 2007). The mean dose received was 3.9 mg/kg bw. Another group of four served as controls. Groups of eight animals were sacrificed at 7, 19, 29, 40, 70 and 77 days after treatment. Mean residues are shown in Table 5.10.

Table 5.10. Monepantel sulphone residues in edible tissues of Merino × Dorset cross-bred lambs after a single oral administration of monepantel

Day	Tissue [mean (n = 8) residues in µg/kg (±SD)]				
	Renal fat	Subcutaneous fat	Liver	Kidney	Muscle
7	3 068 ± 1050	3 667 ± 1316	2 056 ± 733	460 ± 170	155 ± 76

19	681 ± 298	751 ± 364	354 ± 169	99 ± 47	32 ± 11 + 1 at <10 µg/kg
29	83 ± 45	114 ± 91	51 ± 40	18 ± 5 + 5 at <10 µg/kg	All <10 µg/kg
40	22 ± 13 + 2 at <10 µg/kg	21 ± 14 + 2 at <10 µg/kg	18 ± 7 + 1 at <10 µg/kg	All <10 µg/kg	All <10 µg/kg
70	All <10 µg/kg	All <10 µg/kg	11 + 7 at <10 µg/kg	All <10 µg/kg	Not analysed
77	15 + 7 at <10 µg/kg	All <10 µg/kg	12 ± 2 + 2 at <10 µg/kg	Not analysed	Not analysed

Residue depletion in Merino sheep

A residue depletion study with 2–3 year old Merino sheep was reported (Karadzovska, 2007f). The mean oral dose received was 3.8 mg/kg bw. A group of 4 animals served as controls. Animals were sacrificed at 7, 18, 29, 35, 70, 120 and 127 days after treatment; mean tissue residues are shown in Table 5.11.

Table 5.11. Monepantel sulphone residues in edible tissues of Merino sheep after a single oral administration of monepantel

Day	Tissue [mean residues in µg/kg (±SD)]				
	Renal Fat	**Subcutaneous Fat**	**Liver**	**Kidney**	**Muscle**
7	3 109 ± 834	3 010 ± 1028	1 376 ± 258	366 ± 90	199 ± 110
18	474 ± 380	638 ± 497	325 ± 259	82 ± 62	40 ± 39
29	202 ± 132	265 ± 197	138 ± 84	35 ± 12 + 2 at <10 µg/kg	23 ± 6 + 2 at <10 µg/kg
35	67 ± 52	89 ± 46	54 ± 47 +1 at <10 µg/kg	22 ± 6 +6 at <10 µg/kg	15 ± 4 +6 at <10 µg/kg
70	22 ± 10 + 1 at <10 µg/kg	27 ± 15 + 2 at <10 µg/kg	15 ± 6 + 3 at <10 µg/kg	All <10 µg/kg	All <10 µg/kg
120	All <10 µg/kg	All <10 µg/kg	All <10 µg/kg	All <10 µg/kg	All <10 µg/kg
127	All <10 µg/kg	All <10 µg/kg	All <10 µg/kg	Not analysed	Not analysed

Comparison of residue data from all residue studies

Statistical analysis of variance (significance level of 5%) of the data from the three single-dose administration studies indicated that there was no difference in observed residues amongst the three studies. Therefore, data from the above single-dose administration studies were pooled (Karadzovska, 2007f), as shown in Table 5.12.

Table 5.12. Pooled monepantel sulphone residues in edible tissues of sheep

Day	Tissue [mean residues in µg/kg (±SD)]				
	Renal fat	**Subcutaneous fat**	**Liver**	**Kidney**	**Muscle**
7	3 145 ± 962	3 031 ± 1235	1 730 ± 588	472 ± 180	192 ± 101
18/19	548 ± 335	652 ± 391	297 ± 198	84 ± 53	38 ± 26 + 4 at<10 µg/kg
29	134 ± 101 + 1 at <10 µg/kg	169 ± 147	93 ± 70 + 1 at <10 µg/kg	26 ± 12 + 9 at <10 µg/kg	20 ± 6 +15 at <10 µg/kg
35	67 ± 52	89 ± 46	54 ± 47	22 ± 6	15 ± 4

			+ 1 at <10 µg/kg	+ 6 at <10 µg/kg	+6 at <10 µg/kg
40	65 ± 67 + 4 at <10 µg/kg	63 ± 70 + 2 at <10 µg/kg	37 ± 35 + 3 at <10 µg/kg	18 ± 9 + 12 at <10 µg/kg	12 + 15 at <10 µg/kg

The residues were highest in fat, with renal and subcutaneous fat having similar levels, as would be expected for a lipid-soluble molecule, followed by liver, kidney and muscle. The relative magnitude of residues in tissues is about 20:10:2:1, respectively, during early phase depletion (≤2 weeks). Muscle residues presumably arise, in part, from the interstitial fat in muscle tissue. Beyond day 35, the magnitude of liver residues approaches those of fat.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Analytical method for monepantel sulphone residues in sheep tissues

A validated analytical procedure (Karadzovska, 2007b) was used for the quantification of monepantel sulphone residues in edible sheep tissues from the ADME (Karadzovska, 2007g) and residue depletion studies. A ground tissue sample (1 g) was extracted at room temperature with acetonitrile (9 ml), either by mechanical homogenization for 2 minutes or by mechanical shaking for 10 minutes. The mixture was centrifuged briefly, and the supernatant was clarified by syringe filtration prior to dilution. The diluted extract was injected onto HPLC for quantification to an LOQ of 50 µg/kg. When an increased sensitivity was required, the stored extract (prior to filtration) would be cleaned up on a SPE cartridge (either silica based C_{18} for liver, or polymeric for the other tissues). Following evaporation and re-dissolution, an aliquot was injected into the same HPLC system for quantification to an LOQ of 10 µg/kg. The HPLC system was an isocratic 2-column switching system, with a narrow band of eluate from the first column (Luna 3 µm C_{18} (2) silica) being directed to the second column (NovaPak Phenyl 4 µm silica). The same mobile phase (acetonitrile/methanol/water) was used for both columns. The eluate from column 2 was monitored with a UV detector set at 230 nm. The combined retention time was approximately18 minutes. The HPLC was calibrated by injection of pure standards from 2 to 1000 ng/ml.

The method is simple, uses commonly available reagents and without any hazardous steps. The extraction, centrifugation, filtration and dilution steps can be performed by any experienced analyst. Switching HPLC systems, while not common, are easily set-up using readily available HPLC modules plus a switching valve, which can be controlled by instrument software. Up to 18 samples can be processed in a working day and automated HPLC analysis can be performed overnight. The SPE option, however, is more labour-intensive. The method was used extensively in the analysis of samples from several depletion studies and it was determined to be robust and there were no critical control points identified in the procedure.

Confirmation of monepantel sulphone residues was conducted on a separate LC-MS/MS system. The LC-MS/MS system was a gradient system with increasing concentrations of acetonitrile in water, on an Atlantis T3 (C_{18} silica, 3 µm) column. In the enhanced product ion mode, the specific *m/z* ions at 487 and 186 were monitored. Analytical Procedure 272B.00 was validated under GLP conditions.

The performance characteristics of the method including selectivity, accuracy and precision, stability of analyte in solution and under frozen storage conditions, LOD and LOQ were validated as follows.

Selectivity

Numerous drug-free control samples of each tissue type were tested and no interferences were observed at the specified retention time. Similarly, a range of commonly used drugs approved for use in sheep (or their metabolites) were tested, and no interferences were observed. Of the metabolites derived from monepantel, only the parent itself was tested and did not co-elute. The only other major metabolites known to occur in sheep tissues (G32 and sulphoxide M1) will not interfere as their retention differs sufficiently on a C_{18} column, as demonstrated in the pivotal sheep ADME study. The

method is not stereo-specific, so the enantiomer of monepantel sulphone would be detected, but the ADME study demonstrated that racemization does not occur.

Accuracy and precision

Precision and accuracy of the method was tested by analysing batches of 6 replicates at different levels encompassing 0.5 × MRL and 2 × MRL. Mean accuracies were acceptable, within -30 ± 10%. Precision, measured as %CV, were 12% or less for fortifications at 50 µg/kg or higher. Within-laboratory reproducibility was assessed by comparing results obtained by two analysts, each completing a batch of 6 replicates at 10 µg/kg (with SPE) and 50 µg/kg (without SPE) for each tissue type. Mean recoveries were within 9% of each other at 50 µg/kg and within 25% at 10 µg/kg.

Limit of detection (LOD) and limit of quantitation (LOQ)

The calculated LODs and LOQs are based on analyses of drug-free samples. The LODs for the regulatory method option (without SPE cleanup) were 23, 22, 6 and 8 µg/kg for fat, liver, kidney and muscle tissue, respectively. The corresponding LOQs were 51, 56, 13 and 15 µg/kg. For the more sensitive method option that uses an additional SPE cleanup, the LODs were 6, 4, 5 and 5 µg/kg in fat, liver, kidney, and muscle tissues, respectively. The corresponding LOQs were 15, 7, 14 and 10 µg/kg, respectively.

Stability

The stock solution of monepantel sulphone in acetonitrile was stable for 12 months (Karadzovska, 2007g). The corresponding fortification and calibration solutions were also stable for 6 months. The primary extract of tissue samples was stable for 7 days when stored in a refrigerator. Prepared HPLC extracts were stable after 24 h storage at room temperature. A GLP-compliant study investigating the stability of incurred samples under freezer storage conditions, room temperature storage and freeze-thaw (3 cycles) was reviewed. Triplicates of each sample were stored under each of the conditions, and then analysed by the above method (without SPE). After 3 cycles of freeze-thaw, incurred residues of monepantel sulphone were within 94–100% of initial values. After 4 h storage at room temperature, results were within 97–107% of initial values. Samples under freezer storage (-20°C) were analysed after 1.5, 3 and 6 months storage. For liver and fat, there was no change in residue values, but for kidney there was a small decrease (17%) after 6 months storage. Muscle was stable under long-term freezer storage conditions for 1 year.

Analytical method for monepantel and its sulphone in blood

As numerous studies involved analysis of blood samples, the sponsor developed and validated an analytical method for sheep blood (Karadzovska, 2007h; Browning and Karadzovska, 2008). A 0.5 ml aliquot of blood, with anti-coagulant, was extracted with acetonitrile (1.3 ml) and water (0.5 ml) and, following centrifugation, the extract was diluted with water and cleaned-up on a polymeric SPE cartridge. The eluate was evaporated and re-dissolved in mobile phase for HPLC quantification, using the same system as the tissue method, except that a separate switch for monepantel was performed within the same run. The estimated LODs were 0.5 and 0.7 ng/ml for monepantel and its sulphone respectively. The LOQs of 3 ng/ml for each analyte were validated with at least 6 replicate samples showing acceptable accuracy and precision. The method was validated in the range 3 to 1000 ng/ml for both analytes with fortified samples. Accuracy and precision (both repeatability and within-laboratory reproducibility) were excellent. Solutions of the analytes in solvent or mobile phase were stable for 6 months or longer. Prepared blood samples for HPLC injection were stable after storage overnight at room temperature. Blood samples, both fortified and incurred, were stable under 3 cycles of freeze-thaw, after storage at room temperature for 4 h, and after 4 months storage in the freezer (-20°C).

The version of the validated analytical method without SPE, described above, with a LOQ of 50 µg/kg (Browning, 2010), was determined to be a suitable regulatory control method. In addition, a validated LC-MS/MS method is available to confirm the presence of monepantel sulphone residues at the LOQ of 50 µg/kg.

APPRAISAL

Monepantel has not previously been evaluated by the Committee. Monepantel was included in the agenda for the current meeting of the Committee at the request of the 19th Session of the CCRVDF. Monepantel is an anthelmintic of the amino-acetonitrile derivative class indicated for the treatment of nematodes in sheep. The recommended dose is 2.5 mg/kg bw and the maximum dose used is 3.75 mg/kg bw.

Data from two pharmacokinetic GLP-compliant studies were available, one with i.v. administration and another as an oral drench. In both studies, blood samples were collected up to 28 days post-dose. Monepantel and monepantel sulphone concentrations in blood were quantified by a validated HPLC method. In blood and plasma, monepantel concentration decreased rapidly after i.v. administration and was detected until 48 h. Monepantel sulphone concentrations declined in blood and were quantified during 4 days. The oral bio-availability of monepantel was 31%. The area under the curve of monepantel sulphone concentrations obtained after oral administration of monepantel at a dose of 1 mg/kg bw was similar to the value obtained from those given i.v. administration of monepantel sulphone, demonstrating a first-pass effect and complete absorption of the oral dose of monepantel.

In an ADME and residue depletion GLP-compliant study with a single oral dose of ^{14}C-monepantel as a 2.5% (w/v) solution was administered to sheep. Approximately 50% of the administered radio-activity was recovered in faeces and 30% in urine after 14 days. Two different positions of the [^{14}C] labelling in monepantel were used to assess other possible modes of metabolism for the compound. The position of the radiolabel on either ring did not influence the interpretation of the total radio-activity or the metabolic profiling in tissue and excreta. The metabolite profile was analysed and metabolites identified. Monepantel was metabolized to a sulphoxide and a sulphone, identified as the predominant metabolites. A second metabolite pathway involved cleavage to yield the phenol metabolite together with its sulphate conjugate. Monepantel sulphone was the major metabolite found in blood and tissue and represented 100% of radioactivity in blood. Fat was the tissue with the highest concentration of radioactivity followed by liver, kidney and muscle. The approximate total radioactive residue proportions were 10 (fat): 5 (liver): 2 (kidney): 1 (muscle).

In the ADME and residue depletion GLP-compliant study, monepantel and monepantel sulphone were quantified in tissue using a validated HPLC/UV method with a chiral column and a LOQ of 50 µg/kg, and the results were compared. The concentrations of radiolabelled monepantel in the tissue matrices decreased in the order: fat > liver > kidney > muscle. The corresponding concentrations of monepantel sulphone residues decreased in the same order. Total radioactive residue (TRR) expressed as monepantel-equivalent were compared with monepantel and monepantel sulphone tissue concentrations at different time points from 2 to 35 days to calculate the ratio of marker residue and TRR. The ratio of the mean concentration of the marker residue and that of the total residue was calculated as 1 for muscle and 0.66 for fat, liver and kidney.

Three depletion studies were evaluated: one using Suffolk lambs; the second using cross-bred lambs (Merino × Dorset); and the third using Merino sheep. In each study, sheep were administered monepantel at 3.8–3.9 mg/kg bw using the same general study design. Animals were sacrificed at 7, 18, 29, 35, 70, 120 and 127 days after treatment. Residue concentration data from the three single-dose administration studies were statistically compared. An analysis of variance study of the data indicated that there was no significant difference in observed residues amongst the 3 studies at the significance level of 5%. Therefore, the 3 data sets were pooled and used for the estimated daily intake analysis. The median concentration of monepantel sulphone measured in the animal tissues seven days post-dose were 2 620 µg/kg in fat, 1 295 µg/kg in liver, 406 µg/kg in kidney and 152 µg/kg in muscle.

Using the combined data from the three single oral dose monepantel administration studies, the upper one-sided 95% confidence limit over the 95th percentile of residue concentrations was calculated for each edible tissue. The ratio of the mean concentration of the marker residue to that of the TRR was calculated as 1.0 for muscle and 0.66 for fat, liver and kidney. The estimated daily intake (EDI) for monepantel was calculated after applying a correction factor of 0.94 to account for the mass difference between monepantel sulphone (the marker residue) and monepantel (See Figure 5.4). The

time point at which the MRLs were recommended was based on an EDI < ADI approach described in the report of the 66th meeting of the Committee (FAO/WHO, 2006).

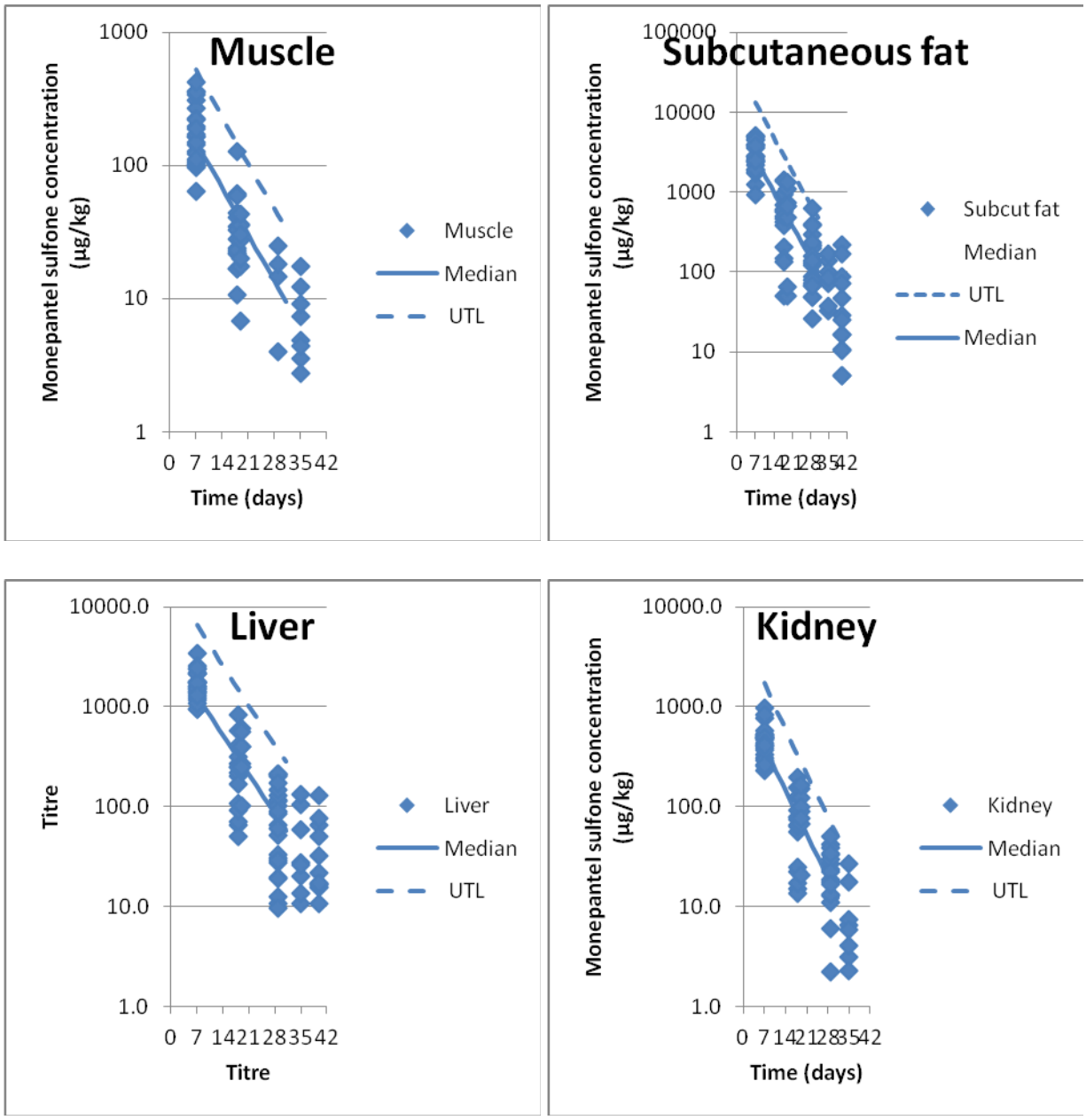


Figure 5.4. Monepantel sulphone residue depletion curves after oral administration of monepantel to sheep (combined data). Median and Upper 95/95 Tolerance Limit (UTL).
NOTE: In the liver figure, the Y-axis is monepantel sulphone (µg/kg) and X- axis is time (days).

A validated HPLC/UV method was available and was used for the analysis of incurred residues of monepantel as its sulphone metabolite in edible sheep tissues. The method provided fit for purpose performances for muscle, kidney, liver and fat samples. Concentrations in tissue samples were determined by reference to non-matrix-matched, external standard calibration curves and can be used for the regulatory monitoring of residues of monepantel and monepantel sulphone in edible tissues. Additionally, a validated LC-MS/MS method suitable for confirming the analytes at 50 µg/kg was available.

MAXIMUM RESIDUE LIMITS

In recommending MRLs for monepantel in sheep, the Committee considered the following factors:

- Monepantel is registered for use in sheep at a maximum recommended single oral dose of 3.75 mg/kg bw.
- An ADI of monepantel 0–20 µg/kg bw was established by the Committee, corresponding to an upper bound of acceptable intakes of 1200 µg/day for a 60 kg person.
- Monepantel is extensively metabolized.
- Monepantel sulphone is the marker residue in tissues.
- Fat contains the highest concentration of monepantel sulphone at all sampling times, followed by liver, then kidney and muscle. Liver and fat can serve as the target tissues.
- The ratios of the concentration of marker residue to total residues are 1.0 in muscle and 0.66 in fat, liver and kidney.
- Residue data evaluated were determined with a validated analytical method to quantify monepantel sulphone in tissue.
- A validated analytical method for the determination of monepantel sulphone in edible sheep tissues (liver, kidney, muscle and fat) is available and may be used for monitoring purposes.
- MRLs were calculated on the basis of the upper limit of the one-sided 95% confidence interval over the 95th percentile of residue concentrations.
- The time point at which the MRLs were set was based on an EDI < ADI approach described in the 66th meeting of the Committee.
- No data are provided for sheep milk.

The Committee recommended MRLs determined as monepantel sulphone in sheep tissue at 300 µg/kg in muscle, 700 µg/kg in kidney, 3000 µg/kg in liver and 5500 µg/kg in fat. Using the model diet and marker to total residue of 1 for muscle and 0.66 for fat, liver and kidney, and after applying a correction factor of 0.94 to account for the mass difference between monepantel sulphone (the marker residue) and monepantel, the EDI is 201 µg/person per day, which represents 17% of the upper bound of the ADI.

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Narasin

First draft prepared by
Bruno Le Bizec, Nantes, France
and
Richard Ellis, Myrtle Beach, USA

Addendum to the monograph prepared by the 70th meeting of the Committee
and published in FAO JECFA Monographs 6

IDENTITY

International Non-proprietary names (INN): Narasin

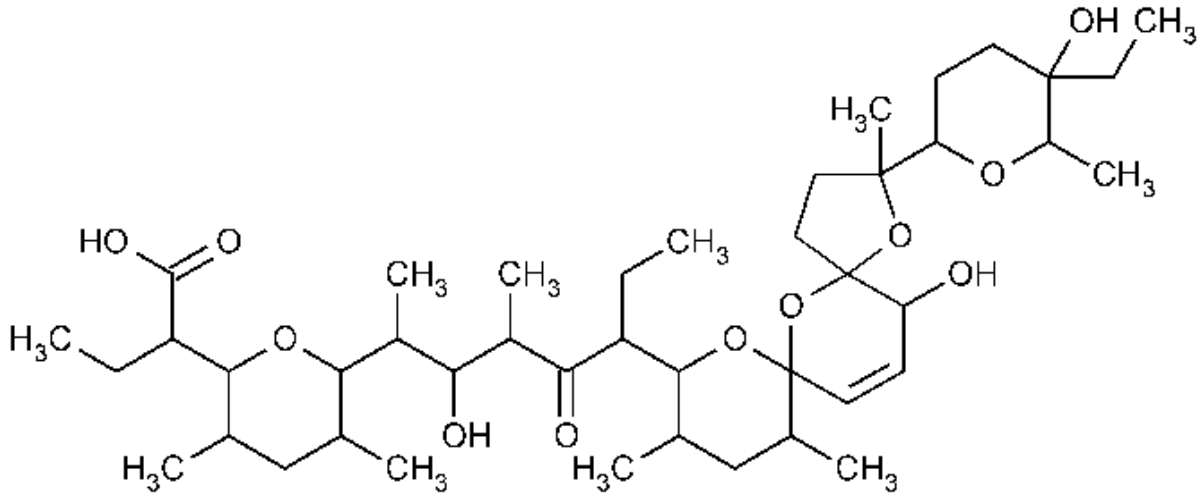
Synonyms: (4s)-4-methylsalinomycin, Narasin A, Monteban®, Naravin®

IUPAC Names: α-ethyl-6-[5-[2-(5 ethyltetrahydro-5-hydroxy-6-methyl-2H-pyran-2-yl)-15-hydroxy-2, 10, 12-trimethyl-1, 6, 8-trioxadispiro [4.1.5.3] pentadec-13-en-9-yl]-2-hydroxy-1, 3-dimethyl-4oxoheptyl] tetrahydro-3,5-dimethyl-2H-pyran-2-acetic acid.

Chemical Abstract Service Number: 55134-13-9

Molecular formula of Narasin A: $C_{43}H_{72}O_{11}$

Molecular mass: 765 g/mol



BACKGROUND

The Committee evaluated the residue safety of narasin in multiple species of food animals at its 70th meeting (FAO, 2009). In the evaluation, the Committee considered narasin A (shown above) to be a suitable marker residue for narasin in animal tissues of cattle, pigs and chickens. At that time, the Committee recommended and CCRVDF concurred, that MRLs for narasin A in cattle tissues (15 µg/kg for muscle and kidney tissues and 50 µg/kg for liver and fat tissues) were temporary as the analytical method was not adequately validated. The Committee requested that before re-evaluation of narasin for consideration of permanent MRLs in tissues of cattle, a detailed description of a suitable regulatory method, including its performance characteristics and validation data, be provided by the end of 2010. The sponsor has prepared and submitted detailed reports on a method validation for the determination of narasin A in cattle tissues.

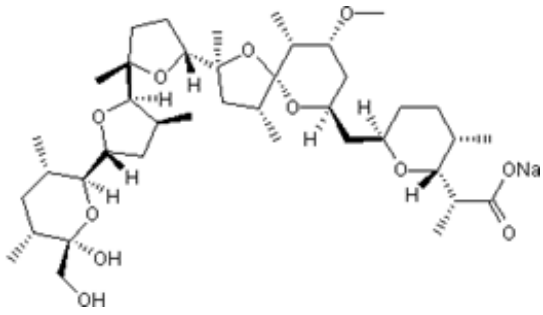
The sponsor submitted three documents in support of the method validation for narasin in cattle tissues. The first is a copy of the method formatted according to the ISO 78/2 format. The second document is a report for a GLP-compliant validation study conducted for monensin A and narasin A in cattle tissues. (MacDougall, 2011a). This study report includes data for monensin A and narasin A, however, only the narasin A data is reviewed here. The third document is a validation data summary for two additional fortification levels in muscle and liver (MacDougall, 2011b). This additional work was conducted to correct an error in the original validation protocol whereby the target concentrations for liver and muscle were transposed and did not correspond to the temporary MRLs. The full dataset is not available for these samples, but the data tables have been fully audited by a quality assurance unit for compliance with GLP. The cattle dataset is a subset of an extensive validation programme in conjunction with the AOAC International that will also include validation data for chicken and pig tissues.

ANALYTICAL METHOD

A method for the determination and confirmation of monensin A and narasin A in cattle tissues and milk was validated (MacDougall, 2011a). The laboratory method (Charles River Method Number 1775 Version 1) was reported as written by Charles River Laboratory in Report Appendix 3 of that report. The method was formatted according to the ISO 78/2 format and assigned Version number 1A (Analytical Method Number 1775). Only the narasin data are relevant to this evaluation.

Sample preparation

Muscle, liver or kidney test samples are initially processed from sample material at approximately -20°C. Initial processing involves homogenizing each test sample with dry ice using a food grinder. Samples are allowed to freeze-dry for 24 h at -20°C before weighing 5 ± 0.1 g tissue samples. Test samples are mixed with iso-octane/ethyl acetate (90:10) followed by agitation using four 10 mm steel balls and mixed for 5 minutes in a high-speed tissue homogenizer (900 shakes/min). Samples are centrifuged for 5 minutes at 3000 rpm (*g* values not reported) at 4°C. Solvent is decanted and the procedure with the high-speed tissue homogenizer is repeated. Combined solvent extracts are mixed with anhydrous sodium sulphate. Dried samples are added to the silica solid phase extraction tubes and eluted with ethyl acetate/methanol (80/20) after pre-treatment of the silica SPE. Extracts are evaporated to dryness under nitrogen, and reconstituted in 1.0 ml methanol for LC-MS-MS analysis. Quantification is from a matrix-matched calibration line and is based on monensin A and narasin A. For fat samples, following treatment with the high-speed tissue homogenizer samples are centrifuged at 4000 rpm for 10 minutes at 20°C, refrigerated at 4°C for approximately 15 minutes, extraction solvent is decanted and the process repeated. The alternative is employed to minimize particulates forming a suspension. Nigercin ($C_{40}H_{67}NaO_{11}$, mol mass: 746.94) is used as an internal standard. The structure is reported below.



Nigercin

Analytical measurement

HPLC separation employs a Phenomenex Aqua® 5 µm C_{18} 150 × 2 mm column with elution at 40°C using a gradient elution mixed phase of 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B). The autosampler is maintained at 4°C and the injection volume is 2 µL. Run time is 10 minutes. The MS-MS analysis employs positive MRM using an electrospray ion source (5500 V). Transitions monitored for quantitative determination of narasin are 787.5 > 431.3 (CE = 67 eV) and confirmation 787.5 > 531.3 (CE = 60 eV) and 787.5 > 279.2 (CE = 73 eV). For the internal standard, nigercin, the transition monitored is 746.6 > 729.6 (CE = 55 eV). The latter is not highly specific (loss of –OH). A splitter system may be used as appropriate to ensure the detector source remains cleaner.

Method performance

System suitability was demonstrated based upon column efficiency, peak width at half height, tailing factor and system precision for each test item. System linearity was demonstrated over the range of 0.5 to 100 µg/kg (all tissues) for matrix-match calibration standards prepared in extracted control samples for each matrix. The accuracy (percent recovery) and precision (CV) determined for three sets of six replicate determinations at three levels are summarized in Table 6.1.

Table 6.1. Accuracy (percent recovery) and precision (CV) for determination of narasin A in cattle tissues at the LOQ (0.75 µg/kg in muscle, liver and kidney; 1.0 µg/kg fat)

Matrix	Narasin A Mean (n = 6)% Recovery (CV%)
Muscle	75.4 (2.88)
Liver	93.9 (6.23)
Kidney	96.4 (5.37)
Fat	88.2 (2.44)

The inter-day assay accuracy and precision was determined for each matrix at their respective ½MRL, MRL and 2MRL levels on three occasions. However, the fortification levels for liver and muscle were transposed during the validation. Additional fortification levels were subsequently validated in a separate study (See below, MacDougall, 2011b). The recoveries and precision (CV) are summarized in Table 6.2.

Table 6.2. Inter-day assay accuracy and precision for the determination of narasin A in cattle tissues on three occasions

Matrix	Temp MRL[2] (µg/kg)	½ × MRL[1]	MRL[1]	2 × MRL[1]
		Mean% Recovery (CV%)	Mean% Recovery (CV%)	Mean% Recovery (CV%)
Muscle	15 (50) [1]	97.8 (5.85)	96.2 (6.65)	99.7 (5.40)
Liver	50 (15) [1]	93.0 (9.55)	95.5 (7.70)	99.4 (5.85)
Kidney	15	87.8 (7.07)	87.2 (6.63)	86.6 (5.92)
Fat	50	81.6 (7.82)	82.0 (6.80)	84.9 (6.55)

NOTES: (1) Fortification levels for liver and muscle were transposed; parenthetical value was actually fortified instead of the MRL. (2) Recommended temporary MRLs at 70th meeting of the Committee.

The assay limit of detection (LOD) and limit of quantitation (LOQ) were determined for narasin A in each matrix, as summarized in Table 6.3.

Table 6.3. Assay limits of detection (LOD) and quantitation (LOQ) demonstrated in tissues fortified with narasin A

Matrix	Narasin A		Ratio LOQ/LOD
	LOD (µg/kg)	LOQ (µg/kg)	
Muscle	0.019	0.75	39.5
Liver	0.038	0.75	19.7
Kidney	0.026	0.75	28.8
Fat	0.151	1.00	6.6

NOTES: Values are rounded

Supplemental validation data for an analytical method for the determination of narasin A in bovine liver, and muscle by LC-MS/MS. Quality assurance verified

As noted above, during the original validation study for cattle tissues (MacDougall, 2011a), the tissue concentrations for the method validation for liver and muscle were transposed, resulting in the incorrect validation range for concentrations of narasin A (See Table 6.2). Additional liver and muscle control samples were fortified and analysed for narasin A using the same method reported in MacDougall, 2011a. The data in the Tables 6.4 and 6.5 summarize the individual data sets for narasin A employing the correct concentrations and provides the intra-day and inter-day accuracy and precision calculations (MacDougall, 2011b).

Table 6.4. Intra-day and inter-day assay accuracy and precision for the determination of narasin A in cattle liver on three independent replicates. (MacDougall, 2011b)

Narasin fortification	Analysis 1		Analysis 2		Analysis 3		Inter-day statistics			
	µg/kg	Recovery (%)	µg/kg	Recovery (%)	µg/kg	Recovery (%)	Mean µg/kg	Recovery (%)	s_r (µg/kg)	RSD_r %
25 µg/kg	21.1	84.4	21.0	84.0	20.0	80.0	20.9	83.8	1.3	6.1
	18.0	72.0	22.2	88.8	20.6	82.4				
	19.6	78.4	22.5	90.0	19.0	76.0				
	22.0	88.0	21.2	84.8	21.5	86.0				
	20.9	83.6	21.3	85.2	22.8	91.2				
	22.6	90.4	20.8	83.2	19.9	79.6				
Mean	20.7	82.8	21.5	86.0	20.6	82.5				
s_r	1.67		0.687		1.35					
RSD_r	8.08		3.20		6.52					
50 µg/kg	37.5	75.0	42.8	85.6	43.1	86.2	42.4	84.7	3.4	8.1
	44.6	89.2	42.0	84.0	44.9	89.8				
	34.8	69.6	39.3	78.6	45.8	91.6				
	38.3	76.6	43.9	87.8	46.1	92.2				
	42.0	84.0	44.0	88.0	44.9	89.8				
	37.8	75.6	44.3	88.6	46.5	93.0				
Mean	39.2	78.3	42.7	85.4	45.2	90.4				
s_r	3.52		1.88		1.22					
RSD_r	8.99		4.41		2.70					
100 µg/kg	79.5	79.5	86.8	86.8	107	107.0	89.2	89.2	7.2	8.1
	90.1	90.1	88.4	88.4	97.7	97.7				
	85.5	85.5	82.2	82.2	91.5	91.5				
	94.7	94.7	91.7	91.7	87.6	87.6				
	85.9	85.9	83.0	83.0	99.9	99.9				
	78.3	78.3	86.5	86.5	88.9	88.9				
Mean	85.7	85.7	86.4	86.4	95.4	95.4				
s_r	6.22		3.51		7.46					
RSD_r	7.26		4.06		7.82					

NOTES: s_r = Standard Deviation. RSD = Reproducability standard deviation

Table 6.5. Intra-day and inter-day assay accuracy and precision for the determination of narasin A in cattle muscle on three independent replicates. (MacDougall, 2011b)

Narasin fortifi-cation (µg/kg)	Analysis 1		Analysis 2		Analysis 3		Inter-day statistics			
	µg/kg	Recover y (%)	µg/kg	Recove ry (%)	µg/kg	Recove ry (%)	Mean (µg/kg)	Recove ry (%)	s_r (µg/kg)	RSD_r %
7.5	7.15	95.3	7.18	95.7	6.82	90.9	7.2	96.4	0.4	5.0
	7.08	94.4	7.57	100.9	6.54	87.2				
	7.01	93.5	7.84	104.5	7.08	94.4				
	7.16	95.5	7.74	103.2	7.29	97.2				
	7.71	102.8	7.23	96.4	7.17	95.6				
	7.17	95.6	7.64	101.9	6.70	89.3				
Mean	7.21	96.2	7.53	100.4	6.93	92.4				
s_r	0.251		0.271		0.292					
RSD_r	3.48		3.59		4.21					
15	16.1	107.3	17.0	113.3	12.2	81.3	14.8	98.6	1.6	10.8
	13.8	92.0	16.9	112.7	13.2	88.0				
	15.4	102.7	17.0	113.3	12.5	83.3				
	14.4	96.0	16.1	107.3	13.1	87.3				
	14.3	95.3	15.3	102.0	13.1	87.3				
	14.7	98.0	16.6	110.7	14.6	97.3				
Mean	14.8	98.6	16.5	109.9	13.1	87.4				
s_r	0.833		0.674		0.828					
RSD_r	5.63		4.09		6.31					
30	30.2	100.7	33.2	110.7	24.2	80.7	28.7	95.7	2.5	8.8
	30.4	101.3	30.6	102.0	26.5	88.3				
	27.4	91.3	31.4	104.7	24.7	82.3				
	29.3	97.7	30.9	103.0	26.3	87.7				
	26.1	87.0	30.9	103.0	28.2	94.0				
	28.5	95.0	31.0	103.3	27.2	90.7				
Mean	28.7	95.5	31.3	104.4	26.2	87.3				
s_r	1.67		0.950		1.51					
RSD_r	5.84		3.03		5.75					

NOTES: s_r = Standard Deviation. RSD = Reproducibility standard deviation

System suitability

The column efficiency, peak width at half height and tailing factor for the test items and internal standards was established. The system precision for reproducibility of response and retention time was determined by replicate injections (n=10) of a standard solution of the test items and internal standard. The precision was defined as the coefficient of variation of the mean value for each parameter.

System linearity

The system linearity was determined by analysing non-extracted matrix-matched standard solutions of known amounts of each test item and the internal standard. Standards were prepared over a range of concentrations of each test item with a fixed concentration of internal standard. The detector response ratio for the test item/internal standard was plotted against the amount injected of the test item to generate a calibration curve. Calculated amounts of the injected standards were determined by using a least squares linear regression analysis with weighting factor of 1/x. The origin was excluded from the

regression analysis. The calculated amount injected for each prepared standard was required to be within ±15% of the actual amount injected (±20% at the lower limit of linearity) to define the linear range of the system.

System limit of detection

The limit of on-column detection (LOOCD) was determined by analysis of solutions of each test item with decreasing concentrations. The LOOCD was defined as the amount injected of each test item that gives a clearly discernible peak, and was at least 3 times greater than the background noise.

Assay limit of quantitation

The limit of quantitation (LOQ) for each test item was determined by the extraction and analysis of replicate aliquots (n=6) of control matrix fortified with decreasing concentrations of each test item, and assaying these samples with the standard method. The target intra-day assay accuracy at the LOQ (defined as the mean percentage measured concentration versus actual concentration) was 70–110%. The precision at each concentration (defined as the CV of the mean concentration) was ≤20%.

Intra-day assay accuracy and precision

The assay accuracy and precision was determined by the extraction and analysis of replicate aliquots (n=6) of each matrix fortified with each test item, together with a non-extracted matrix-matched calibration curve series of standard solutions. Samples were prepared at ½MRL, MRL and 2MRL for each matrix. The target intra-day assay accuracy at each concentration (defined as the mean percentage measured concentration versus actual concentration) was 70–110%. The target intra-day assay precision at each concentration (defined as the CV of the mean concentration) was ≤20%.

Inter-day assay accuracy and precision

The assay accuracy and precision was determined on three occasions, as detailed for the intra-day assay accuracy and precision. The target inter-day assay accuracy at each concentration (defined as the mean percentage determined concentration/actual concentration of all the replicate samples from all three occasions) was 70–110%. The target inter-day assay precision at each concentration (defined as the CV of the mean requirement concentration) was ≤20%. To demonstrate the ruggedness of the assay, at least one of the occasions was extracted by a second analyst.

Assay specificity

The specificity of the assay for each test item and the internal standard was examined by extraction and analysis of aliquots of each matrix with and without the addition of the test item. The assay requirement for each test item was “no significant interfering substances >20%” (a very permissive value) of peak area at LOQ level eluting at the same retention times as the test items or the internal standard. The following analytes were also used to evaluate the potential for interference: penicillin, tylosin, tilmicosin, tetracycline, lasalocid, ceftiofur, ractopamine and ketoprofen. This study was limited to injection of solution standards.

Assay limit of detection

The assay limit of detection (LOD) was determined by extraction and analysis of 20 aliquots (4 extractions from each of 5 different animals) of the matrix to determine the mean background noise. The LOD was defined as the concentration of each test item equivalent to the mean background noise plus 3 times the standard deviation.

Storage stability at room temperature

Storage stability was demonstrated to be acceptable for each matrix at room temperature (about 4 h) and for extended frozen storage (approximately -20°C) up to 2 months. The stability of samples after three freeze-thaw cycles was demonstrated for each matrix. The stability of extracts stored at about 4°C was shown to be approximately 72 h for each matrix.

The effects of storing samples at room temperature was investigated by preparing replicate matrix samples fortified with each test item at the relative MRL concentrations, followed by storage at room

temperature for about 4 h prior to extraction (representative of the actual times necessary to prepare and analyse a batch of samples prior to extraction). To define the reference (initial) concentrations, replicate aliquots (n=6) of the matrix was prepared (fortified with solutions prepared independently from the calibration standard solutions) and assayed. The mean calculated concentration of the replicate samples was determined and was defined as the reference (i.e. 100%) concentration. Acceptance criteria for reference concentration samples were the same as for assay accuracy and precision analysis. The stability of the test items was defined as the mean 4 h post-storage concentration/reference concentration (expressed as a percentage) for that level. The test items were considered to be stable in the matrix at room temperature if the stability was 100 ± 20%.

Freeze-thaw stability

The effects of repeatedly freezing and thawing samples were investigated by preparing replicate matrix samples fortified with each test item at the relative MRL, and repeatedly freezing and thawing prior to extraction. To define the reference (initial) concentrations, replicate aliquots (n=6) of the matrix were prepared (fortified with solutions prepared independently from the calibration standard solutions) and assayed. The mean calculated concentration of the replicate samples was determined and defined the initial concentration. Acceptance criteria for reference concentration samples were the same as for assay accuracy and precision analysis.

To determine the freeze-thaw stability of the test item in the matrix, 3 sets of replicate aliquots (n=6) of the matrix were prepared (fortified with solutions prepared independently from the calibration standard solutions). Following fortification of the tissues the samples were stored frozen for a minimum of 24 h. The samples were then thawed until they reach room temperature, and then re-frozen for a minimum of 24 h. Samples were subjected to 1, 2 or 3 freeze-thaw cycles. The samples were assayed together with a non-extracted matrix-match calibration curve and freshly prepared samples at the same fortified fortification level after the appropriate number of cycles.

The stability of each test item for each freeze-thaw cycle was defined as the mean post freeze-thaw concentration/concentration of the fresh extracts expressed as a percentage for that number of cycles. The test items were deemed to be stable in the matrix if the stability was 100 ± 20%.

Autosampler stability

The effect of storing extracts of samples was investigated by extracting replicate matrix samples fortified with each test item at the MRL and 2MRL and storing at +4°C for about 72 h prior to analysis. Replicate aliquots (n=6) of the matrix were prepared at each concentration (fortified with solutions prepared independently from the calibration standard solutions) and extracted, together with a non-extracted matrix-matched calibration curve series of standard solutions. The calibration samples and the replicates at each concentration were analysed before being stored at about +4°C for about 72 h prior to analysis. This was representative of the actual times necessary to prepare and analyse a batch of samples and to permit a repeat analysis (without re-extraction), if required, and then assayed. The mean post-storage calculated concentrations of the replicate samples at each level were determined. The stability was defined with respect to the recovery data prior to the circa 72 h storage period. The autosampler storage was considered to be acceptable if the recoveries were 100 ± 20% of the samples analysed immediately.

Solution stability

The stability of selected calibration solutions of each test item was investigated by periodically re-preparing standard and QC solutions and analysing them together with a non-extracted calibration series of standards previously prepared. The solutions were deemed to be stable for a period corresponding to difference in time between the preparations of the two sets of standard solutions if the mean accuracy of freshly prepared solutions (defined as the mean percentage determined concentration/actual concentration) was 100 ± 20%.

Extended frozen storage stability

The effects of storing samples frozen at about -20°C were investigated by preparing samples fortified with each test item at the MRL in each matrix. Sufficient samples were prepared in each matrix to

permit replicate samples (n=6), which were had not been thawed since preparation, to be taken at each time point. To define the reference (initial) concentrations, replicate aliquots (n=6) of the matrix were prepared (fortified with solutions prepared independently from the calibration standard solutions) and assayed. The mean calculated concentration of the replicate samples was determined and was defined as the initial concentration. Acceptance criteria for reference concentration samples were the same as for assay accuracy and precision analysis.

To determine the storage stability of the test item in the matrix at about -20°C after approximately 1 and 2 month frozen storage, replicate samples (n=6) were thawed and assayed together with a non-extracted matrix-match calibration curve and freshly prepared fortified samples. The stability of the test item was defined as the percentage difference between the freshly fortified extracts on each occasion and the time points T=1 and 2 months. The test item was deemed to be stable in the matrix under frozen conditions if the stability was 100 ± 20% of the fresh extracts.

Assay acceptance criteria

For the analysis of the test items in bovine tissues described above, the following additional criteria were met. The determined concentration for each prepared non-extracted matrix-match (injected at the front and the end of any batch) standard used to construct the calibration curve was within 100 ± 15% of the actual concentration (100 ± 20% at the lower limit of quantitation). At least 75% of the calibration standards met the above criteria.

Statistical analysis

Statistical analysis was limited to derivation of means, standard deviations, coefficient of variation and regression parameters.

Means, standard deviations and precisions (CV%).

Table 6.6 provides assay limits of quantitation; Tables 6.7–6.10 provide accuracy and precision data in fortified tissues; and Figures 6.1–6.4 describe matrix match calibration lines in control tissue.

Table 6.6. Narasin A assay limits of quantitation

Analyte	Fortification level (µg/kg)	Recovery (%)	Mean recovery (%)	CV (%)
In Muscle				
Narasin	0.75	71.9 76.6 76.5 77.0 77.0 73.5	75.4	2.88
In Liver				
Narasin	0.75	88.0 86.7 94.5 101 99.7 93.7	93.9	6.23
In Kidney				
Narasin	0.75	101 98.9 96.7 98.4 86.3 96.8	96.4	5.37
In Fat				
Narasin	1.00	86.1 91.5 87.6	88.2	2.44

89.0
85.8
89.3

Table 6.7. Assay accuracy and precision in muscle tissue fortified at 25 µg/kg with Narasin A

			Intraday		Interday	
Fortification Level (µg/kg)	Occasion	Recovery (%)	Mean Recovery (%)	CV (%)	Overall Mean Recovery (%)	Overall CV (%)
25	1	102	99.9	6.36	97.8	5.85
		105				
		94.4				
		98.6				
		108				
		91.3				
	2	97.3	97.6	3.74		
		90.6				
		98.1				
		98.7				
		101				
		99.7				
	3	86.1	96.1	7.27		
		93.9				
		103				
		102				
		101				
		90.4				

Table 6.8. Assay accuracy and precision in liver tissue fortified at 30 µg/kg with Narasin A

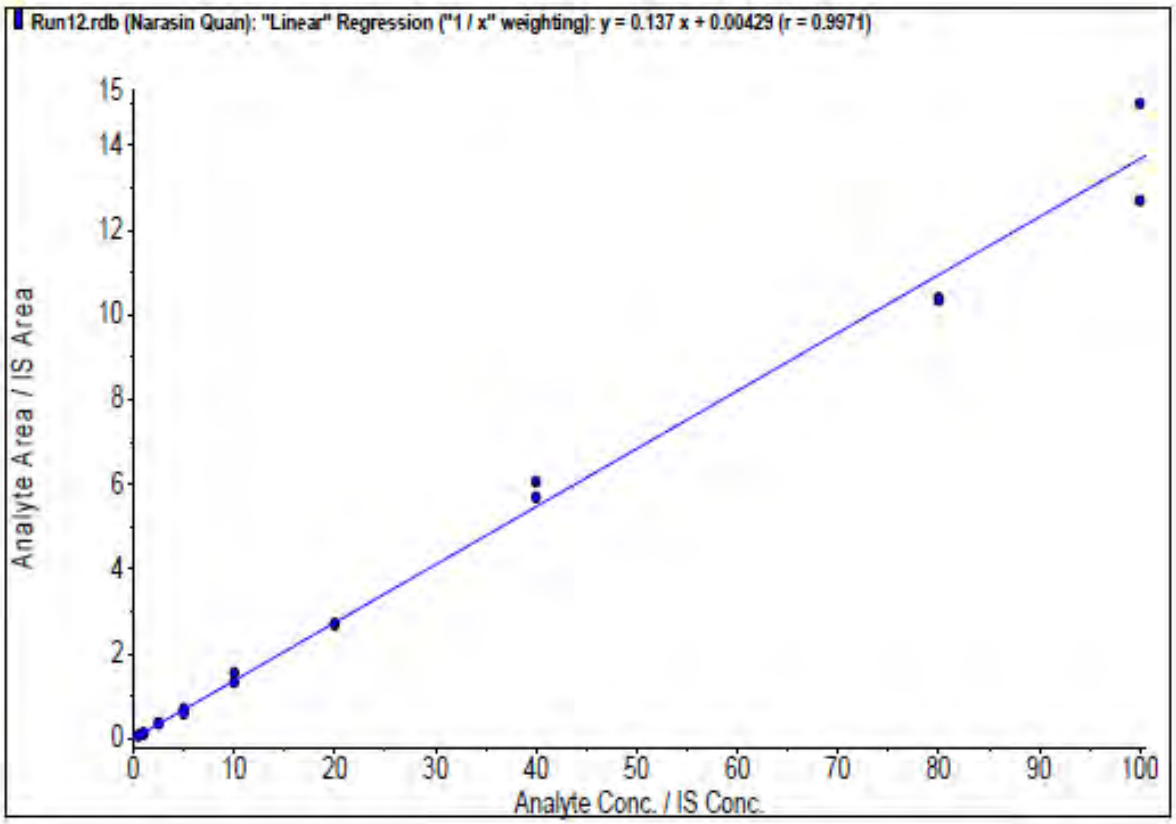
			Intraday		Interday	
Fortification Level (µg/kg)	Occasion	Recovery (%)	Mean Recovery (%)	CV (%)	Overall Mean Recovery (%)	Overall CV (%)
30	1	98.0	104	5.17	99.4	5.85
		105				
		98.8				
		108				
		112[A]				
		103				
	2	98.0	99.6	4.54		
		94.7				
		101				
		103				
		95.0				
		106				
	3	92.8	94.3	2.91		
		93.1				
		97.2				
		98.2				
		91.2				
		93.4				

Table 6.9. Assay accuracy and precision in kidney tissue fortified at 15 µg/kg with Narasin A

			Intraday		Interday	
Fortification Level (µg/kg)	Occasion	Recovery (%)	Mean Recovery (%)	CV (%)	Overall Mean Recovery (%)	Overall CV (%)
15	1	93.0	92.1	4.98	87.2	6.63
		88.8				
		93.4				
		98.7				
		93.5				
		85.3				
	2	83.1	87.0	4.67		
		90.8				
		86.5				
		92.9				
		85.9				
		82.9				
	3	77.1	82.5	5.44		
		83.0				
		84.1				
		79.9				
		90.1				
		80.5				

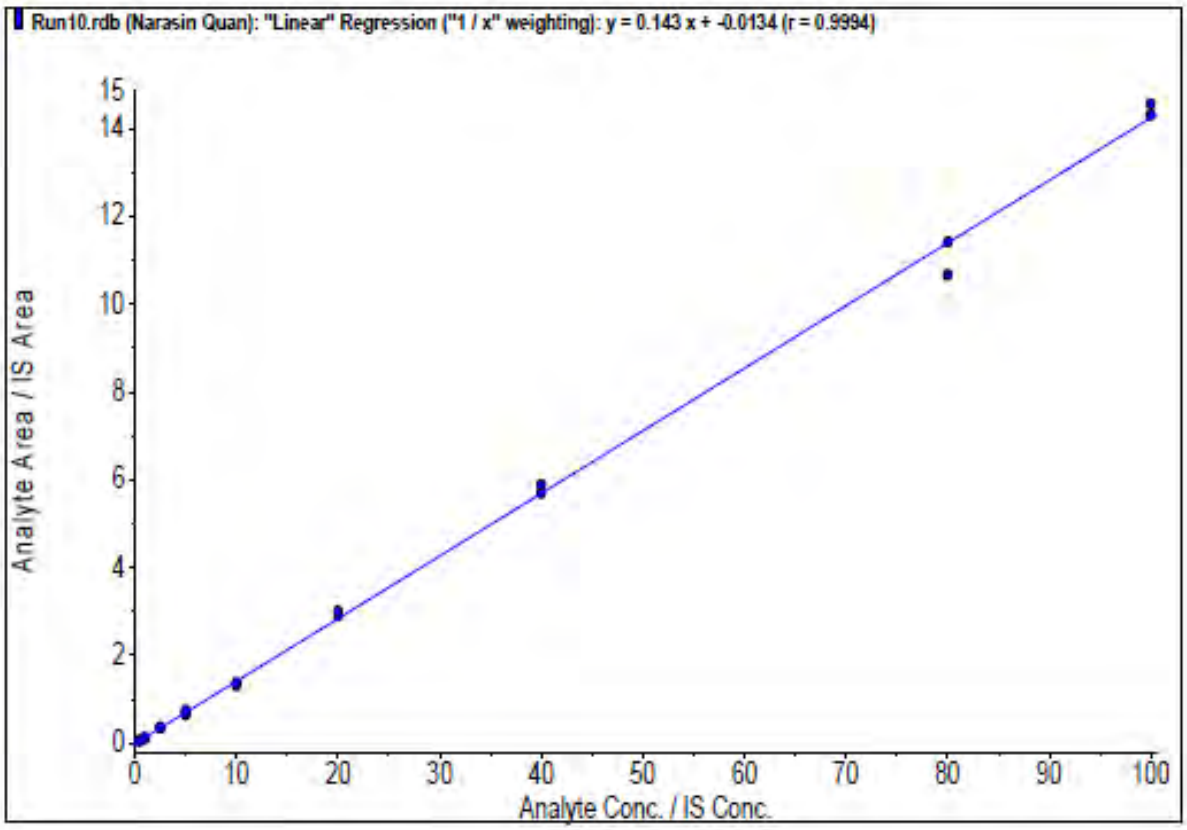
Table 6.10. Assay accuracy and precision in fat tissue fortified at 50 µg/kg with Narasin A

			Intraday		Interday	
Fortification Level (µg/kg)	Occasion	Recovery (%)	Mean Recovery (%)	CV (%)	Overall Mean Recovery (%)	Overall CV (%)
50	1	81.4	83.8	3.41	82.0	6.80
		81.7				
		83.4				
		88.8				
		82.0				
		85.3				
	2	83.0	85.7	3.69		
		84.7				
		88.4				
		83.4				
		83.7				
		90.7				
	3	73.6	76.7	7.63		
		85.3				
		72.7				
		80.5				
		69.4[A]				
		78.7				



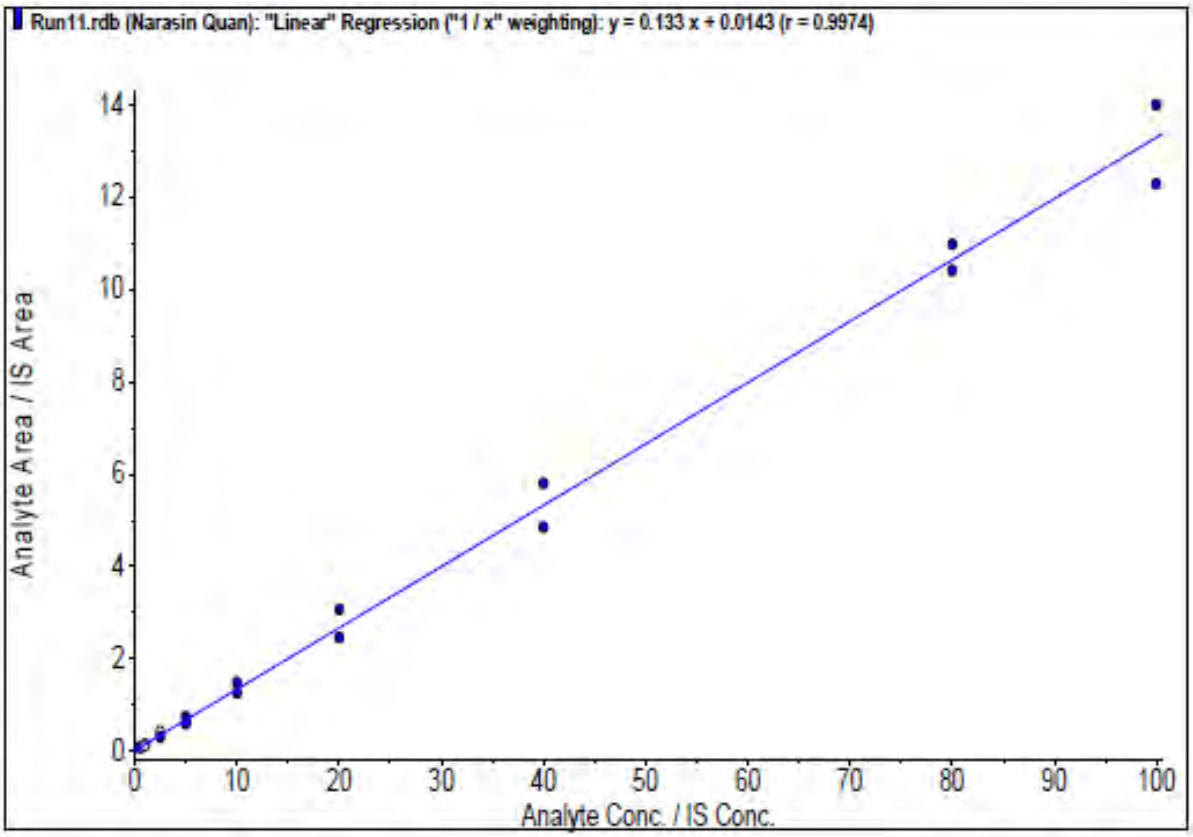
		Front Calibration Line		Back Calibration Line	
Standard ID	Actual Concentration (ng/mL)	Found Concentration (ng/mL)	Accuracy (%)	Found Concentration (ng/mL)	Accuracy (%)
MM L	0.5000	0.454	90.8	0.509	102
MM K	1.000	0.765	76.5 [A]	0.937	93.7
MM J	2.500	2.73	109	2.56	103
MM I	5.000	5.16	103	4.27	85.5
MM H	10.00	11.3	113	9.63	96.3
MM G	20.00	19.6	98.0	19.9	99.4
MM F	40.00	41.6	104	44.3	111
MM E	80.01	76.0	95.0	75.6	94.5
MM D	100.0	110	110	92.8	92.8

Figure 6.1. Narasin matrix-match calibration line in control muscle extracts (0.5–100 ng/ml)



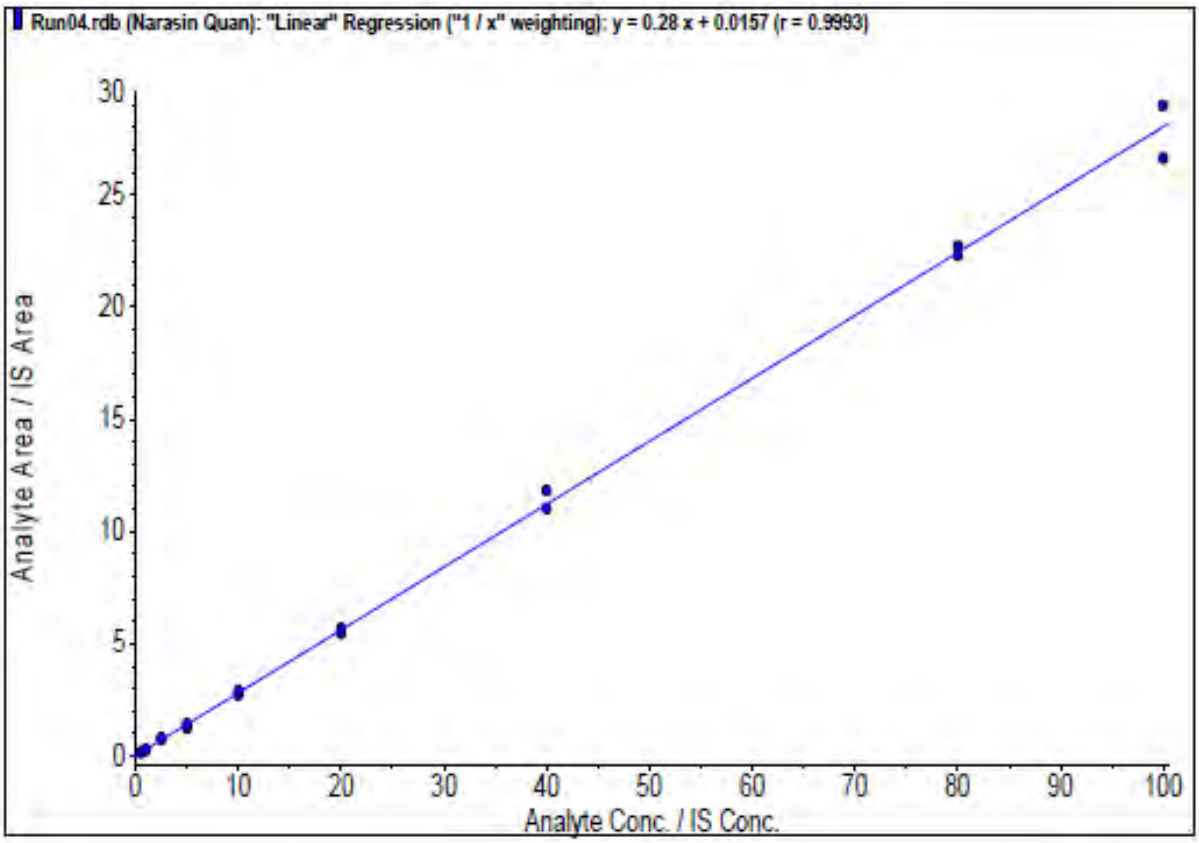
Standard ID	Actual Concentration (ng/mL)	Front Calibration Line		Back Calibration Line	
		Found Concentration (ng/mL)	Accuracy (%)	Found Concentration (ng/mL)	Accuracy (%)
MM L	0.5000	0.512	102	0.507	101
MM K	1.000	0.911	91.1	0.968	96.8
MM J	2.500	2.62	105	2.62	105
MM I	5.000	4.65	93.0	5.35	107
MM H	10.00	9.36	93.6	9.73	97.3
MM G	20.00	20.5	102	21.1	106
MM F	40.00	40.1	100	41.4	103
MM E	80.01	74.9	93.6	80.2	100
MM D	100.0	100	100	102	102

Figure 6.2. Narasin matrix-match calibration line in control liver extracts (0.5–100 ng/ml)



Standard ID	Actual Concentration (ng/mL)	Front Calibration Line		Back Calibration Line	
		Found Concentration (ng/mL)	Accuracy (%)	Found Concentration (ng/mL)	Accuracy (%)
MM L	0.5000	0.496	99.3	0.509	102
MM K	1.000	1.03	103	0.794	79.4 ^
MM J	2.500	3.07	123 ^	2.23	89.1
MM I	5.000	5.46	109	4.38	87.5
MM H	10.00	11.1	111	9.39	93.9
MM G	20.00	23.0	115	18.4	91.9
MM F	40.00	43.6	109	36.4	91.1
MM E	80.01	78.3	97.8	82.5	103
MM D	100.0	92.4	92.4	105	105

Figure 6.3. Narasin matrix-match calibration line in control kidney extracts (0.5–100 ng/ml)



		Front Calibration Line		Back Calibration Line	
Standard ID	Actual Concentration (ng/mL)	Found Concentration (ng/mL)	Accuracy (%)	Found Concentration (ng/mL)	Accuracy (%)
MM L	0.5000	0.486	97.1	0.512	102
MM K	1.000	0.896	89.6	0.995	99.5
MM J	2.500	2.62	105	2.88	115
MM I	5.000	4.43	88.6	5.08	102
MM H	10.00	9.66	96.6	10.3	103
MM G	20.00	19.5	97.3	20.2	101
MM F	40.00	39.3	98.3	42.2	105
MM E	80.01	81.0	101	79.6	99.4
MM D	100.0	95.0	95.0	103	103

Figure 6.4. Narasin matrix-match calibration line in control fat extracts (0.5–100 ng/ml)

APPRAISAL

The 70th meeting of the Committee reported on the availability of screening, quantitative and confirmatory methods for narasin in chicken, pig and cattle tissues that may be appropriate for regulatory control programmes (FAO/WHO, 2009). GLP-compliant screening methods were reported based on extraction followed by thin layer chromatography-bioautography methods (Maruyama and Sugimoto, 2000). The bioautography was performed by melting agar over the surface of the TLC plate seeded with *Bacillus stearothermophilus* var. *calidolactis* C-953 inoculum. The limit of quantitation (LOQ) was estimated to be 25 µg/kg. Calibration curves showed good linearity within the tested concentrations of 0.1–3.2 mg/kg. However, the accuracy, precision and the limit of detection (LOD) of the assay were not given. In another GLP-compliant screening study (Handy, Thomson and Tamura, 1985), a TLC-bioautographic method, using *Bacillus subtilis* as the indicator organism, was described. The limit of detection was 5 µg/kg.

A Time-Resolved Fluorescence Immunoassay (TR-FIA) screening method for the detection of narasin was developed in a non-GLP compliant study (Peippo *et al.,* 2004). Muscle tissue extracts were applied to a microtitre well containing an antibody (goat anti-sheep IgG), and an aliquot of unlabelled narasin-transferrin conjugate in a reconstitution buffer. The time resolved fluorescence was measured by a multi-label counter. The LOD of this method was 560 µg/kg, the LOQ was 800 µg/kg. The results of the precision intra-assay and inter-assay were 3.5 and 3.6% (CV) respectively.

Confirmatory methods for narasin were also reported using HPLC methods with UV_{vis} detection using chromatographic analysis post-column derivatization with vanillin reagent that produces a product that absorbs at 520 nm. (Ward *et al.,* 2005; Lacoste and Larvor, 2003). Different authors have described the use of LC coupled to mass spectrometry to determine narasin in edible chicken tissues. The analyses are performed in the positive ion electrospray modes. The mass spectrometric methods are suitable and provide better specificity and sensitivity than do the HPLC-UV methods. Because the methods require only a simple extraction with a short run time (about 12 minutes), large-sample batches (more than 20 samples) can be processed daily.

However, the 70th meeting of the Committee noted that suitable analytical methods have been described for the determination and confirmation of narasin only in edible tissues of chickens and pigs. Residues in cattle could only be determined using a TLC-bioautographic method. This method, while having a reported test sensitivity of 5 µg/kg, had results of residue values reported only as a range (e.g. 10–20; 5–10). As a result, only temporary MRLs for cattle were recommended by the Committee using the LOQ values for the HPLC-UV methods.

The sponsor has provided a new GLP-compliant HPLC-MS/MS method. Three documents were submitted in support of the method validation for narasin in cattle tissues: a copy of the method formatted according to the ISO 78/2 format; a report for a GLP-compliant validation conducted for monensin A and narasin A in cattle tissues; and a validation data summary for two additional fortification levels in muscle and liver. This additional work was conducted because the original validation protocol transposed concentrations for liver and muscle temporary MRLs. The full dataset was not available for these samples, but the data tables have been fully audited by a quality assurance unit for compliance with GLP. The cattle dataset is a subset of an extensive validation programme in conjunction with the AOAC International that will include validation data for chicken and pig tissues as well. While not noted in the report, the method has been developed for narasin and monensin in tissues of cattle, chickens and pigs.

The reports document acceptable system suitability, system linearity, accuracy and precision, limits of detection and quantitation, but do not specifically specify the limit of identification, although expected to be consistent with the LOQ. The LOD was determined to be 0.026–0.151 µg/kg for the four primary tissues and the LOQ was determined to be 0.75 µg/kg for muscle, liver and kidney tissues and 1.0 µg/kg for fat tissue. All values are well below the recommended temporary MRLs from the 70th meeting of the Committee. Other performance factors demonstrating method performance and method validation include intra- and inter-day accuracy and precision performance, analytical specificity with a number of veterinary antibiotic drugs, analyte-fortified storage stability, freeze-thaw

and extended frozen storage stability, autosampler stability and solution stability. The method description, reagents, equipment, mass spectrometry settings and conditions are adequately described. Data provided should enable a regulatory laboratory to develop specific quality control and quality assurance documents to support laboratory and regulatory control use.

MAXIMUM RESIDUE LIMITS

In recommending MRLs for narasin in cattle, the Committee considered the following factors:

- A new GLP-compliant validated HPLC-MS/MS complete with adequate performance factors and method validation was provided that was considered suitable for routine monitoring for narasin A as marker residue.
- The analytical method has been validated for use in cattle tissues and is also appropriate for chicken and pig tissues.

The 70th meeting of the Committee recommended temporary MRLs of 50 µg/kg for cattle liver and fat, and 15 µg/kg for cattle muscle and kidney, determined as narasin A. The LOQs for the new analytical method for cattle tissues are more than adequate to accommodate the MRLs recommended at the 70th meeting of the Committee for other animal species and tissues.

The Committee recommended full MRLs for narasin of 15 µg/kg for cattle muscle and kidney, and 50 µg/kg for liver and fat tissues, determined as narasin A.

The 70th meeting of the Committee decided not to calculate the Estimated Daily Intake because there were insufficient data points in the residue depletion studies to calculate the median values for residues. Using the model diet and a marker:total residue ratio of 5%, the MRLs recommended above would result in a theoretical maximum daily intake of 255 µg per person per day, which represents approximately 85% of the upper bound of the ADI.

REFERENCES

Charles River Laboratory. 2011. Analytical method for the determination of Monensin A and Narasin A in bovine liver, kidney, muscle, fat and Monensin A only in bovine milk by LC-MS/MS. Sponsor submitted.

FAO. 2009. Residue evaluation of certain veterinary drugs. 70th Meeting of the Joint FAO/WHO Expert Committee on Food Additives. *FAO JECFA Monographs*, 6. See pp. 137–158.

FAO/WHO. 2009. Evaluation of certain veterinary drug residues in food. Seventieth report of the Joint FAO/WHO Expert Committee on Food Additives. *WHO Technical Report Series,* 954.

Handy, P.R., Thomson, T.D. & Tamura, R.N. 1985**.** Determination of the depletion of narasin residues in broiler chickens. Agricultural Analytical Chemistry, Lilly Research Laboratories, Division of Eli Lilly and Company, Report Number AAC-8408. Sponsor submitted.

Lacoste, E. & Larvor, A. 2003. Residue study in edible tissues of broiler chickens fed with narasin at 80 ppm for five consecutive days. European Animal Science Research. Elanco Animal Health, Division of Eli Lilly and Company, Report Number T2NAFR0103. Sponsor submitted.

Maruyama, N. & Sugimoto, T. 2000. Narasin residue trial in broiler chicken-I. Research Institute for Animal Science in Biochemistry and Toxicology, Lilly Japan K.K, Division of Eli Lilly and Company, Report Number T2NJA9837. Sponsor submitted.

MacDougall, J. 2011a. Narasin Final Report. Validation of an analytical method for the determination of Monensin A and Narasin A in bovine liver, kidney, muscle, fat and milk by LC-MS/MS. Test Facility Study Number 217751. Report No. 31841. Sponsor submitted.

MacDougall, J. 2011b. Charles River Study 218561. Non-clinical laboratory study (GLP): Single laboratory validation of an analytical method for determination of Monensin and Narasin in tissue and milk by LC-MS/MS. Sponsor submitted.

Peippo, P., Hagren, V., Lovgren, T. & Tuomola, M. 2004. Rapid time-resolved fluoroimmunoassay for the screening of narasin and salinomycin residues in poultry and eggs**.** *Journal of Agricultural and Food Chemistry,* 52: 1824–1828.

Ward, T.L., Moran, J.W., Turner, J.M. & Coleman, M.R. 2005. Validation of a method for the determination of narasin in the edible tissues of chickens by liquid chromatography. *Journal of AOAC International,* 88: 95–101. Sponsor submitted.

Triclabendazole

First draft prepared by
Dieter Arnold, Berlin, Germany

Addendum to the monographs prepared by the 40th, 66th and 70th Meetings of the Committee and published in *FAO Food & Nutrition Paper* 41/5 and *FAO JECFA Monographs* 2 and 6, respectively

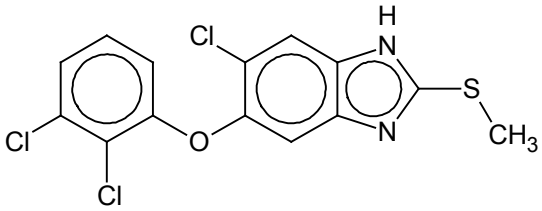
IDENTITY

IUPAC name: 6-chloro-5-(2,3-dichlorophenoxy)-2-methylsulfanyl-1H-benzimidazole

Synonyms: Triclabendazole (common name); CGA 89317, CGP 23030; Proprietary names Fasinex®, Soforen®, Endex®, Combinex®, Parsifal®, Fasimec®, Genesis®, GenesisTM Ultra®.

Structural formula:

Benzimidazoles normally undergo an intermolecular proton transfer between N-1 and N-3 in the imidazole ring which at room temperature is very rapid and yields to tautomers (Iddon *et al.,* 1992).



Molecular formula: $C_{14}H_9C_{l3}N_2OS$

Molecular weight: 359.66 g/mol

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Triclabendazole

Appearance: White crystalline solid

Melting point: The melting point of commercial products is frequently given as 175–176°C. This is also the value given in the Merck Index. Another source: (http://www.wolframalpha.com/entities/chemicals/triclabendazole/b0/ve/bh/) reports 177°C. Another form with a melting point of 85–90°C has also been described in the literature (Iddon *et al.,* 1992). Tothadi *et al.* (2012) describe two anhydrous forms, one consisting of one tautomer only and exhibiting the higher melting point of 177°C and the other consisting of an equimolar mixture of the two tautomers and exhibiting a melting point of 166°C.

Solubility: Soluble in tetrahydrofuran, cyclohexanone, acetone, iso- propanol, n-octanol, and methanol; slightly soluble in dichloro-methane, chloroform, toluene, xylene, ethyl acetate; insoluble in water, hexane.

Octanol water partition coefficient (P_{OW}): 1.75×10^6

UV_{max}: Approximately 305 nm, depending on solvent (Shrivastava, Kumar and Jain, 2011).

RESIDUES IN FOOD AND THEIR EVALUATION

The Committee has reviewed triclabendazole at its 40th, 66th and 70th meetings (FAO/WHO, 1993, 2006, 2009). At the 40th meeting the Committee established an ADI of 0–3 μg/kg bw (0–180 μg/day for a 60 kg person) and recommended Maximum Residue Limits expressed as 5-chloro-6-(2',3'-dichlorophenoxy)-benzimidazole-2-one for muscle, liver, kidney and fat of cattle and sheep. The marker residue on which the MRLs proposed by the 40th meeting were based is produced when

common fragments of triclabendazole-related residues are hydrolysed under alkaline conditions at 90–100°C. Its concentrations can be converted into triclabendazole equivalents by multiplying with a conversion factor of 1.09. The 66th meeting defined the marker residue as "keto-triclabendazole" and recommended MRLs for muscle, liver, kidney and fat in cattle, sheep and goat. The sponsor correctly defined the marker residue as "sum of the extractable residues that may be oxidised to keto-triclabendazole". This definition was also used by the 70th meeting of the Committee: "The marker residue is the sum of all residues extracted and converted to keto-triclabendazole." On this basis the Committee recommended the MRLs listed in Table 7.1.

Table 7.1 MRLs for triclabendazole recommended by the 70th meeting of the Committee

Species	MRL (µg/kg)			
	Muscle	Liver	Kidney	Fat
Cattle	250	850	400	100
Sheep	200	300	200	100

The MRLs in muscle, liver and kidney of both species were derived from the curve describing the upper one-sided 95% confidence limit over the 95th percentile of the residues on day 28 after the last treatment. MRLs for fat were based on twice the LOQ of the analytical method. The MRLs previously recommended by the sixty-sixth meeting of the Committee for triclabendazole for cattle and sheep were withdrawn. As the Committee recommended significantly different MRLs for cattle and sheep, and upon reviewing the limited database for residues in goats, the Committee concluded that there was insufficient data to extend the recommended MRLs for goats. The MRLs for goats recommended at the sixty-sixth meeting of the Committee were withdrawn.

The Committee was requested to review triclabendazole by the 19th session of the CCRVDF, that had raised the specific question: "Can MRLs for goat (tissues) be established by extrapolation considering data used for recommending MRLs for cattle and sheep (tissues)." On the question of data , the CCRVDF had stated: "JECFA has established MRLs for sheep and cattle and extrapolation would be based on the data packages available to the 70th JECFA and literature review to be provided by the United States of America."

Since the dossier provided for evaluation by the 70th meeting has been extensively reviewed by the Committee, the present addendum focuses mainly on studies possibly suitable to answer the question raised by the CCRVDF. These are primarily studies that could be useful for extrapolation from sheep to goat. Some other available studies performed in cattle and sheep are not reviewed again in the present addendum.

Conditions of use

Table 7.2 summarizes recently received information on approved products that are regulated in different countries and which contain triclabendazole and include goat as target species.

Table 7.2. A selection of commercially available triclabendazole products for use in goat

Country	Product	TCBZ	Target animal	Dose	Withdrawal time
Australia	Fasinex 50 Flukicide	5%	cattle		
			sheep		
			goat	1 ml/5 kg	21 days
	Fasinex 100 Oral Flukicide	5%	cattle		
			sheep	1 ml/10 kg	21 days
			goat		
	Young's Tricla 50 Flukicide	5%	cattle		
			sheep	1 ml/5 kg	21 days
			goat		
France	Fascinex 5%	5%	sheep	1 ml/5 kg	28 days
			goat		
Mexico	Fasinex 10%	10%	cattle		
			sheep	1 ml/10 kg	28 days
			goat		
	Fasimec	12% plus 2% ivermectin	cattle		
			sheep	0.5 ml/5 kg	21 days
			goat		
New Zealand	Fasinex 10	10%	cattle		
			sheep	1 ml/10 kg	28 days
			goat		
South Africa	Endex 19.5%	12% plus 7.5% levamisole	cattle		
			sheep	1 ml/10 kg	28 days
			goat		
	Fasinex 10%	10%	cattle		
			sheep	1 ml/10 kg	28 days
			goat		
Switzerland	Fasinex 5%	5%	sheep		
			goat	1 ml/5 kg	28 days

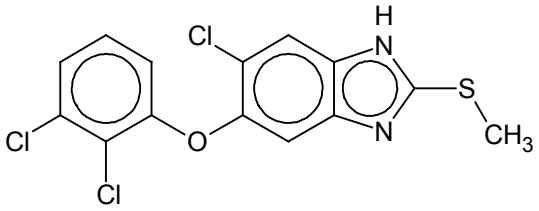
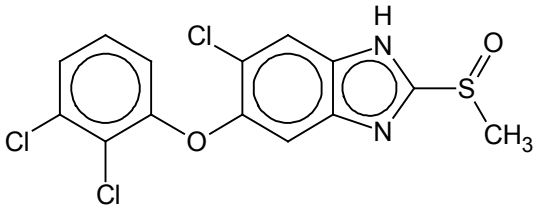
NOTES: TCBZ = triclabendazole; percentages are given on a weight/volume basis.

Dosage

Triclabendazole is typically administered orally to sheep and goat. The recommended dose is usually 10 mg/kg bw, occasionally 12 mg/kg bw in both sheep and goat. The dose is typically administered in liquid formulations (tablets are also available). Body weights are typically rounded up in steps of 5 or 10 kg when calculating the volume of the formulation to be administered. Overdosing may therefore occur systematically, and would be more significant in animals with comparatively low body weights.

PHARMACOKINETICS AND METABOLISM

The following scheme shows some structures related to triclabendazole (metabolites and conversion products) that will be discussed in subsequent paragraphs.

<table>
<tr>
<td>TCBZ
 
6-chloro-5-(2,3-dichlorophenoxy)-2-(methylthio)-1H-benzimidazole
Molecular Formula = $C_{14}H_9Cl_3N_2OS$
Formula Weight = 359.66
Synonyms and abbreviations: CGA 89317 and CGP 23030.</td>
<td>TCBZ-sulphoxide
 
6-chloro-5-(2,3-dichlorophenoxy)-2-(methylsulfinyl)-1H-benzimidazole
Molecular Formula = $C_{14}H_9Cl_3N_2O_2S$
Formula Weight = 375.66
Synonyms and abbreviations: CGA 110752</td>
</tr>
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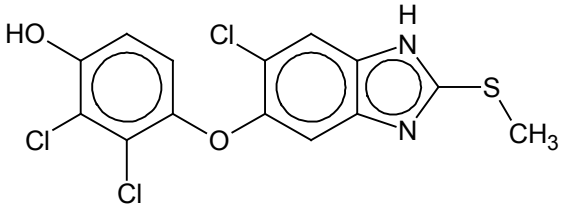
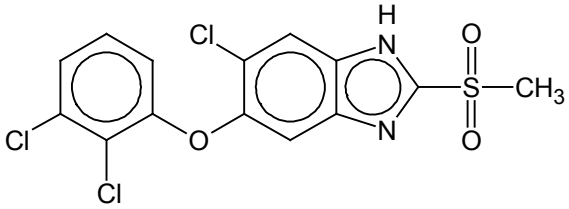
<td>TCBZ-OH
 
2,3-dichloro-4-{[6-chloro-2-(methylthio)-1H-benzimidazol-5-yl]oxy}phenol
Molecular Formula = $C_{14}H_9Cl_3N_2O_2S$
Formula Weight = 375.66
Synonyms and abbreviations: CGA 161944</td>
<td>TCBZ-sulphone
 
6-chloro-5-(2,3-dichlorophenoxy)-2-(methylsulfonyl)-1H-benzimidazole
Molecular Formula = $C_{14}H_9Cl_3N_2O_3S$
Formula Weight = 391.66
Synonyms and abbreviations: CGA 110753</td>
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 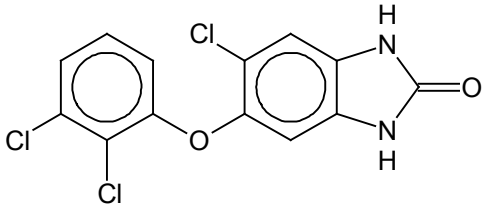
5-chloro-6-(2,3-dichlorophenoxy)-1,3-dihydro-2H-benzimidazol-2-one
Molecular Formula = $C_{13}H_7Cl_3N_2O_2$
Formula Weight = 329.56
Synonyms and abbreviations: CGA 110754</td>
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 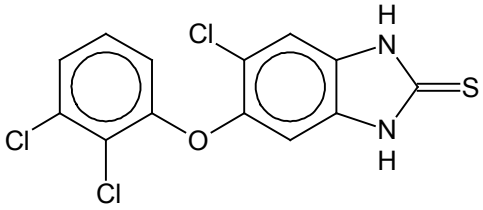
5-chloro-6-(2,3-dichlorophenoxy)-1,3-dihydro-2H-benzimidazole-2-thione
Molecular Formula = $C_{13}H_7Cl_3N_2OS$
Formula Weight = 345.63
Synonyms and abbreviations: CGA 77336</td>
</tr>
</table>

Food Producing Animals

The absorption, distribution, metabolism and excretion are very similar in the laboratory animals and food producing animals studied. A detailed review of the available information was performed by the 70th meeting of the Committee (FAO/WHO, 2009). Some studies are summarized here because the data could be helpful in considerations of between-species extrapolations (sheep and goat).

Sheep and goat

The distribution, degradation and excretion of ^{14}C-labelled triclabendazole were studied in a single female sheep (Hamböck and Strittmatter, 1982) and in a single lactating goat (Hamböck and Strittmatter, 1981). The same radiolabel preparation was used in both animals. The kinetic changes of the concentrations of radioactivity in blood, blood cells (only a few samples) and in plasma were measured during a period of 10 days. During the same period excretion in urine and in faeces (and in milk for goat) was determined. Animals were killed 10 days after the treatment and radioactivity was measured in a great number of tissues including, liver, kidney, muscle and fat. The tissues of both animals were also used to characterize the radioactive residues in these tissues (Hamböck, 1982). The metabolic fate of triclabendazole was finally summarized (including results of studies in the rat and analyses of metabolites in urine and faeces of sheep, goat, and rat) and a metabolic pathway was proposed (Hamböck, 1983). Table 7.3 summarizes the conditions of the experiments performed in the sheep and goat.

Table 7.3. Summary of the studies performed in a single sheep and a single goat

Species and breed	Animal	Age	Body weight	Product used	Formulation	Route	Dosage
Sheep Swiss White Alp × Ile de France	1 female	4 months	28.5 kg	^{14}C-CGA 89317 Specific activity 7.7 µCi/mg	2 gelatin capsules rinsed with ca. 0.5 L water	oral	10.5 mg/kg b w
Goat Chamoises des Alpes	1 female, lactating	Approx. 3 years	42.5 kg		3 gelatin capsules rinsed with ca. 0.5 L water	oral	10.1 mg/kg b w

Figure 7.1 shows the kinetics of the radioactivity in blood and plasma of the two animals and in milk obtained from the goat. The highest concentrations in plasma were measured in the samples taken 24 h after treatment. Concentrations in sheep plasma were higher than in goat plasma and the rates of distribution and elimination were apparently lower in the sheep.

More than 100% of the administered radioactivity was recovered during the observation period of 240 h. Most of the administered radioactivity was excreted in the faeces. The time course of cumulative recovery from urine and faces is shown in Figure 7.2. The curves were very similar in both animals. Cumulative excretion of the radioactivity is given in Table 7.4.

Table 7.4. Recovery of radioactivity in excreta of a sheep and a goat

Animal	Recovery of radioactivity (% of dose)			
	Urine	Faeces	Milk	Total recovery
Sheep	3.54	100.85		104.39
Goat	2.12	98.8	0.56	101.48

Faeces and urine pooled from both animals and over the period 0–72 h were analysed for radioactive metabolites of the parent drug. Using a variety of extraction procedures, chromatographic separations, chemical transformations and physical-chemical identification methods, several metabolite fractions could be separated and some of them could be identified. The pattern of metabolites was qualitatively and quantitatively similar in the two animals.

It was concluded that the predominant pathway of biotransformation was the oxidation of the 2-thiomethyl group producing the sulphoxide and the sulphone, and to a limited extent the 2-benzimidazolone. A separate minor oxidative pathway leads to the 4'-hydroxy derivative (Hamböck, 1983). Several groups of authors have studied the metabolic pathways of triclabendazole. Virkel *et al.* (2006), for example, have shown that both flavin-containing mono-oxygenases (FMO) and cytochromes P450 are involved in the oxidation of triclabendazole in sheep liver. The FMO system is

mainly involved in the sulphoxidation. Both enzyme systems participate in similar proportion in the formation of the sulphone.

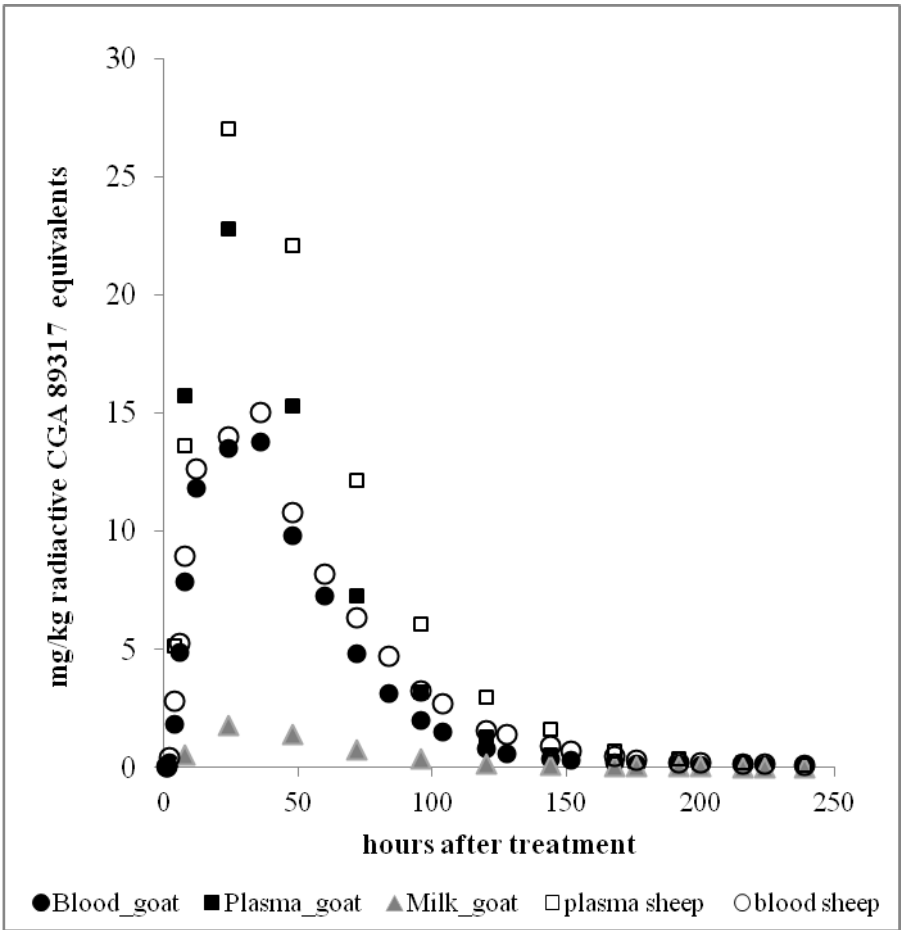

Figure 7.1. Time course of the changes of concentrations of the radioactivity in some body fluids of a sheep and a goat

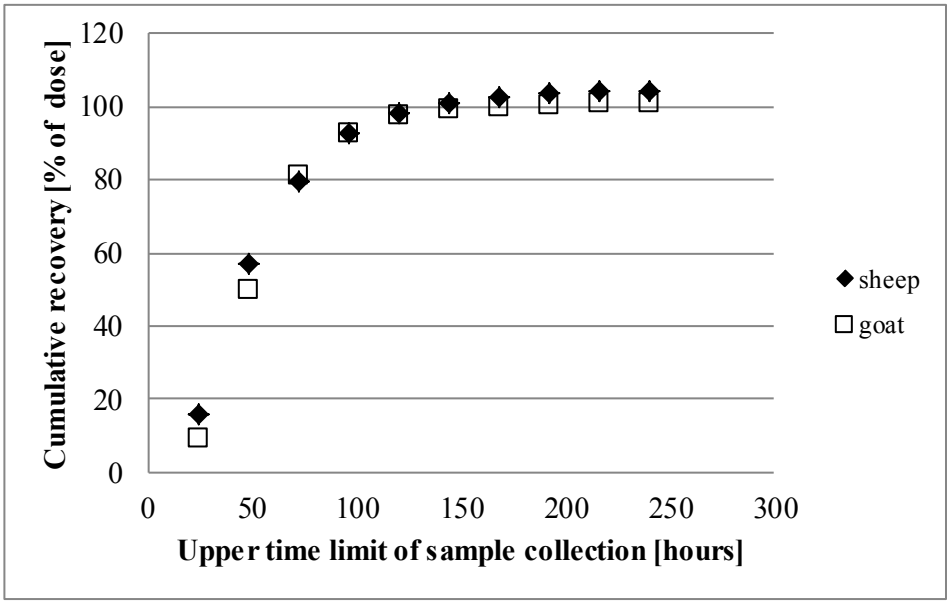

Figure 7.2. Cumulative recovery of the administered radioactivity in urine and faeces of a sheep and of a goat

The total residue equivalents calculated from the radioactivity found in tissues was higher in the tissues of the sheep than in those of the goat; the difference was particularly significant in liver and kidney (see Table 7.5), although the proportions of the concentrations of residues in liver, kidney, muscle and fat were similar in the two animals.

Table 7.5. Total radioactivity in selected tissues of a sheep and of a goat

Tissue		Sheep	Goat
		Total residue (mg/kg)	
Liver		1.84	1.00
Brain		0.95	0.79
Heart		0.92	0.73
Kidney		1.11	0.69
Muscle	rump	0.58	0.44
	round steak	0.58	0.59
	tenderloin	0.53	0.45
Lung		0.35	0.34
Rumen	wall	0.21	0.23
Intestine	wall	0.17	0.15
Thymus		0.11	0.11
Fat	perirenal	0.09	0.08
	subcutaneous	0.08	0.07

The radioactivity in sheep and goat tissues was not readily extractable into solvent systems. Alkaline solubilization of the tissues rendered 72–95% of the radioactivity into a form that could be partitioned into methylene chloride at pH <3 (Hamböck, 1982). Oxidation using hydrogen peroxide transformed 31–45% of the total radioactive tissue residues into the benzimidazole-2-one derivative CGA 110754 (“keto-triclabendazole”). The percentage CGA 110754 found using this procedure was 42% and 40% in muscle of the sheep and the goat, respectively; 45% and 36% in brain of sheep and goat, respectively; and 31% in the lungs of both animals.

Kinabo and Bogan (1988) studied the pharmacokinetics and efficacy of triclabendazole in normal goats (breed, age and body weight not given) and in goats with induced fascioliasis. The drug was administered orally (12 mg/kg bw). Plasma was collected over a period of seven days. Six weeks later the animals were infected and another six weeks later the kinetic experiment was repeated. Samples were analysed for the parent drug, the sulphoxide and the sulphone. The authors state that the observed differences in kinetic parameters (e.g. lower C_{max}, longer t_{max}, longer terminal half-life in infected animals) were not statistically significant. Although there are some discrepancies between the numerical and the graphical presentation of the data in this publication, it is evident that t_{max} of the concentration of the sulphone was almost twice as long as t_{max} of the concentration of the sulphoxide. This finding is consistent with the proposed metabolic sequence. The parent drug was not detected in any sample (LOD = 0.02 μg/ml). Qualitatively similar kinetic patterns of the same two metabolites were observed in a study of Sanyal (1994) after intraruminal administration of 10 mg/kg bw of triclabendazole to five goats (breed, age and body weight not given). In this study, a group of five sheep (breed, age and body weight not given) was also treated in the same way. The differences observed in the kinetic parameters of goat and sheep were considered statistically insignificant. A graph of the data shows that the observation period was probably too short to reliably estimate the half-life of the terminal elimination. The best-fitting curves of the computer modelling were not shown. The parent drug was not detected at any time (LOD = 0.02 μg/ml).

Gokbulut *et al.* (2007) found that the type of diet could have statistically significant effects on the kinetic parameters of the sulphoxide and of the sulphone in plasma of goats. The authors used 5–6-month-old goats (breed not given) weighing 15–18 kg. Two groups of six randomly allocated animals (similar mean body weight in each group) were formed. One group was kept indoors and fed concentrate plus hay rations; the other group was grazed outside. Following three weeks during which the groups were kept on their respective diets, all animals were treated orally with 10 mg/kg bw of triclabendazole. Plasma was obtained from blood samples taken up to 192 h after treatment. Parent drug and the sulphoxide and sulphone metabolites were determined, and the concentration vs time curve of each animal was analysed with the WinNonlin (4.1) software. Pharmacokinetic parameters for the two diets were compared by one-way ANOVA and a value of $P<0.05$ was considered significant. Triclabendazole was found at very low concentrations (t_{max} 12 h) during the first 20 h after treatment. All estimated parameters of the kinetics of the metabolites were significantly different between the two groups. The sulphoxide reached higher concentrations and was less rapidly metabolized to the sulphone in “indoor” animals. Also, fasting of goats for 24 h before and 6 h after treatment resulted in significantly higher absorption and systemic bio-availability of the drug and its metabolites (Gokbulut *et al.,* 2010).

Pharmacokinetic parameters that were determined in all the above studies are summarized in Table 7.6. The only investigation that compared the pharmacokinetics in sheep and goat (Sanyal, 1994) used the intraruminal route for administration (triclabendazole is more typically administered by mouth to sheep and goat). The number of animals used in the study (n=5) was very small for a comparative study. The observation period was too short to adequately cover the terminal elimination (e.g. in the case of the sulphone in sheep plasma, there were only two measurements made after the calculated t_{max}). The author does not indicate for which time period the AUC was determined. The author used a program “PHARMKIT”. Such a program could not be found in the literature. The model parameters of the non-linear curve fitting are not given. Best fitting curves are not shown in the graph. Individual animal data are not provided, so the results cannot be independently verified. The author stated: “There were no differences in C_{max}, t_{max}, AUC and $t_{1/2}$ for each metabolite between the groups.” This statement is not plausible in some cases, particularly when variability within a group was very low and differences between groups were rather large (e.g. some AUC values seem to be significantly different). The author also stated: “Comparison between groups were carried out using t-test”. It is not clear how these tests were calculated (e.g. in cases of unequal variances). There are also some discrepancies between the graphical presentation of the results and the calculated parameters. The results of the other studies performed with goat showed great variability and dependencies on several factors such as dietary conditions of the investigated animals.

Pharmacokinetic interactions between triclabendazole and other drugs were studied by some groups. Lifschitz *et al.* (2009) administered triclabendazole and ivermectin intravenously to Corriedale

sheep with body weights of 20–30 kg, either alone or in combination. A two-compartment model and the software PK Solution® 2.0 was used for curve fitting. Higher C_{max} of the sulphoxide and the sulphone metabolites were observed in the presence of ivermectin.

Triclabendazole was administered intra-ruminally to male Corriedale sheep (14–16 months old, body weight 53.8 ± 2.6 kg, artificially infected with triclabendazole-resistant *F. hepatica*). No statistically significant changes in the pharmacokinetic behaviour of the metabolites of triclabendazole were seen between a group receiving triclabendazole alone and another group which received ivermectin in addition to triclabendazole, applied by s.c. injection, and methimazole given by i.m. injection (Ceballos *et al.,* 2010). It is difficult to interpret the discrepancies between the results of the two studies because too many potentially influential factors were different in the experimental design. It cannot generally be concluded from the results reported by Ceballos *et al.* (2010) that ivermectin has no influence on the pharmacokinetics of triclabendazole.

Inhibition of cytochrome P450 enhances the systemic availability of triclabendazole metabolites in sheep (Virkel *et al.*, 2009). The authors carried out pharmacokinetic studies in Corriedale × Merino weaned female lambs (18.6 ± 4.2 kg). Four treatment groups of five animals each were formed and treated intravenously with either triclabendazole alone or in combination with the FMO inhibitor methimazole (MTZ), or the P450 inhibitors piperonyl butoxide (PB) or ketoconazole (KTZ; this substance was administered orally). Pharmacokinetic data analysis was carried out with PK Solution® 2.0. Methamizole, which inhibits the formation of both the sulphoxide and the sulphone *in vitro,* had no influence under the experimental conditions of the study. Co-administration of PB drastically enhanced the AUC of both the sulphoxide and the sulphone. KTZ is known to inhibit several P450 subspecies. It also enhanced C_{max} and AUC of the metabolites in this study.

The variability of the array of parameters listed in Table 7.6 underlines the difficulty of comparing the results of such studies performed in different laboratories under different experimental conditions. The interesting results of these studies could not be used for between-species extrapolations. At the present time it cannot be excluded that the kinetic behaviour of triclabendazole is different in sheep and goat, and that the drug and/or its metabolites exhibit different kinetic behaviour in certain commercially available combination products.

Table 7.6. Summary of pharmacokinetic parameters of metabolites of triclabendazole in sheep and goats

Treatment group	Dose	Additional conditions	C_{max} (µg/ml)	t_{max} (h)	AUC (µg/h/ml)	Elimination $t_{½}$ (h)	Source
Triclabendazole sulphoxide (Mean ± s.d.)							
5 sheep	10	Stall-fed, intraruminal dose	8.59 ±0.50	32.89 ±0.21	682.75 ±2.42	32.37 ±1.72	Sanyal, 1994
5 goats			10.34 ±0.60	27.82 ±0.38	760.97 ±3.91	32.18 ±1.32	
5 goats	12	Not infected	14.88 ±2.0	12.80 ±1.29	606 ±79	22.38 ±0.66	Kinabo and Bogan, 1988
		Artificially infected	12.99 ±1.2	17.60 ±2.99	490 ±55	23.53 ±3.23	
6 goats	10	Kept Indoors	13.22 ±2.8	18.40 ±2.19	613 ±137	24.77 ±1.92	Gokbulut *et al.,* 2007
6 goats		Grazing	10.17 ±1.5	14.00 ±2.19	406 ±98	16.16 ±1.17	
4 goats	10	Fed	6.49 ±1.7	34.00 ±15.14	376.34 ±51.65	26.96 ±14.98	Gokbulut *et al.,* 2010
4 goats		Fasted	12.98 ±5.4	29.00 ±6.00	654.14 ±171.32	20.93 ±3.67	
5 sheep	5	TCBZ only, i.v.	12.6 ±4.6	2.80 ±1.05	297 ±74.3	16.7 ±4.71	Lifschitz *et al.,* 2009
5 sheep		TCBZ + IVM, i.v.	23.2 ±7.7	1.50 ±0.68	319 ±70.2	10.8 ±1.03	
5 sheep	5	TCBZ only, i.v.	12.9 ±4.4	2.80 ±1.05	296.6 ±76[(1)]	15.6 ±1.77	Virkel *et al.,* 2009
5 sheep		TCBZ, KTZ i.v..	12.0 ±1.1	2.60 ±1.34	253.5 ±96.1	12.2 ±4.27	
5 sheep		TCBZ, PB, i.v.	20.9 ±2.7	5.00 ±2.00	592.5 ±145.2	17.8 ±2.00	
5 sheep		TCBZ i.v., KTZ oral	17.7 ±3.6	4.00 ±3.67	418.7 ±66.4	17.4 ±6.81	
Triclabendazole sulphone (Mean ± s.d.)							
5 sheep	10	Stall-fed, intraruminal dose	7.95 ±0.4	78.1 ±0.22	1449.6 ±3.38	71.7 ±2.13	Sanyal, 1994
5 goats			10.81 ±0.4	59.08 ±0.24	1356.0 ±6.59	54.18 ±2.89	
5-goats	12	Not infected	12.37 ±1.2	25.60 ±1.94	730 ±99	19.36 ±1.11	Kinabo and Bogan, 1988
		Artificially infected	12.11 ±2.1	34.80 ±5.49	699 ±114	21.80 ±2.29	
6 goats	10	Kept Indoors	11.66 ±2.5	44.80 ±7.16	890 ±214	29.75 ±1.91	Gokbulut *et al.*, 2007
6 goats		Grazing	15.05 ±4.9	40.00 ±8.76	1108 ±445	21.43 ±2.00	
4 goats	10	Fed	6.45 ±1.2	56.00 ±11.31	533.93 ±114.05	34.15 ±12.96	Gokbulut *et al.,* 2010
4 goats		Fasted	12.07 ±5.7	52.00 ±4.62	882.93 ±370.26	27.04 ±5.83	
5 sheep	5	TCBZ only, i.v.	7.00 ±1.8	21.6 ±5.26	438 ±85.8	29.6 ±11.4	Lifschitz *et al.,* 2009
5 sheep		TCBZ + IVM, i.v.	10.4 ±2.1	16.0 ±7.35	489 ±116	16.3 ±2.31	
5 sheep	5	TCBZ only, i.v.	7.02 ±1.9	21.6 ±5.37	420.1 ±103.6[(2)]	24.3 ±5.22	Virkel *et al.,* 2009
5 sheep		TCBZ, KTZ i.v.	5.05 ±0.6	21.6 ±10.0	309.1 ±107.3	19.1 ±5.56	
5 sheep		TCBZ, PB, i.v.	11.4 ±1.9	30.0 ±6.93	643.0 ±151.2	23.5 ±6.76	
5 sheep		TCBZ i.v., KTZ oral	8.32 ±1.8	26.4 ±13.2	517.6 ±166.4	24.7 ±10.1	

NOTES; All doses are in mg/kg bw; TCBZ = triclabendazole; IVM = ivermectin; MTZ = methimazole; PB = piperonyl butoxide; KTZ = ketoconazole. (1) All AUC values in this study are for 0–120 h. (2) All AUC values in this study are for 0–144 h.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabelled residue depletion studies

Sheep and goat

No kinetic residue depletion studies with radiolabelled triclabendazole have been reported. The ratio of marker (keto-triclabendazole) to total residue concentrations can only be estimated from some experiments performed with single animals, including those of the Hamböck studies, and of another very limited study (Ferguson, 1994). In the latter study, two sheep, a 27 kg female and a 33 kg male received a dose of 10 mg/kg bw, orally by syringe and gavage tube. They were terminated 28 days later. The composition of the residues in muscle and liver of the male animal was determined in a separate study. The ratio of marker to total residue concentrations re-calculated from the data was 0.40 for muscle and 0.25 for liver. Table 7.7 summarizes the ratios of marker to total residue concentrations for sheep and goat. Data for bovine animals are added for comparison from the corresponding table of the triclabendazole residue monograph of the 70th meeting of the Committee (FAO, 2009).

Table 7.7. Summary of available information on the ratio of marker to total residue concentrations in ruminants

Animal (sex)	Body weight	Dose (mg/kg bw)	Days after dosing	Ratio of marker to total residue concentrations			
				Liver	Kidney	Muscle	Fat
1 Calf (m)	96 kg	12	28	0.19	0.24	0.41	No data
1 Calf (m)	91 kg	12.55	28	0.24	0.27	0.32	No data
1 Sheep (m)	33 kg	10.45	28	0.25	No data	0.4	No data
1 Sheep (f)	28.5 kg	10.5	10	No data	No data	0.42	No data
1 Goat (f)	42.5 kb	10.1	10	No data	No data	0.4	No data

Residue depletion studies with unlabelled drug

Sheep

The kinetic residue depletion studies carried out with sheep and with unlabelled drug have been described in detail in the residue monograph of the 70th meeting of the Committee (FAO, 2009). The most relevant study for the calculation of MRLs by the 70th meeting of the Committee was the study by Adams (2004). These studies were conducted using a commercial formulation. Using these data, MRLs for liver, kidney and muscle were derived from the curves describing the upper one-sided 95% confidence limits over the 95th percentiles of the concentrations of the marker residue on day 28 after the last treatment. Figure 7.3 shows a modification of a graph published in the residue monograph of the 70th meeting of the Committee. It shows, as an example, the abovementioned tolerance limit curves for liver, kidney and muscle of sheep, as well as the data points for muscle on which the tolerance limit curve was based and, in addition, two single data points available for the marker residue concentration in muscle of a single goat and of a single sheep used in the studies of Hamböck and Strittmatter (1981, 1982) and Hamböck (1982, 1983). These two additional points are within the range of expected concentrations on the basis of the data of the Adams (2004) study.

Goat

No residue depletion study was carried out with the unlabelled drug in goat.

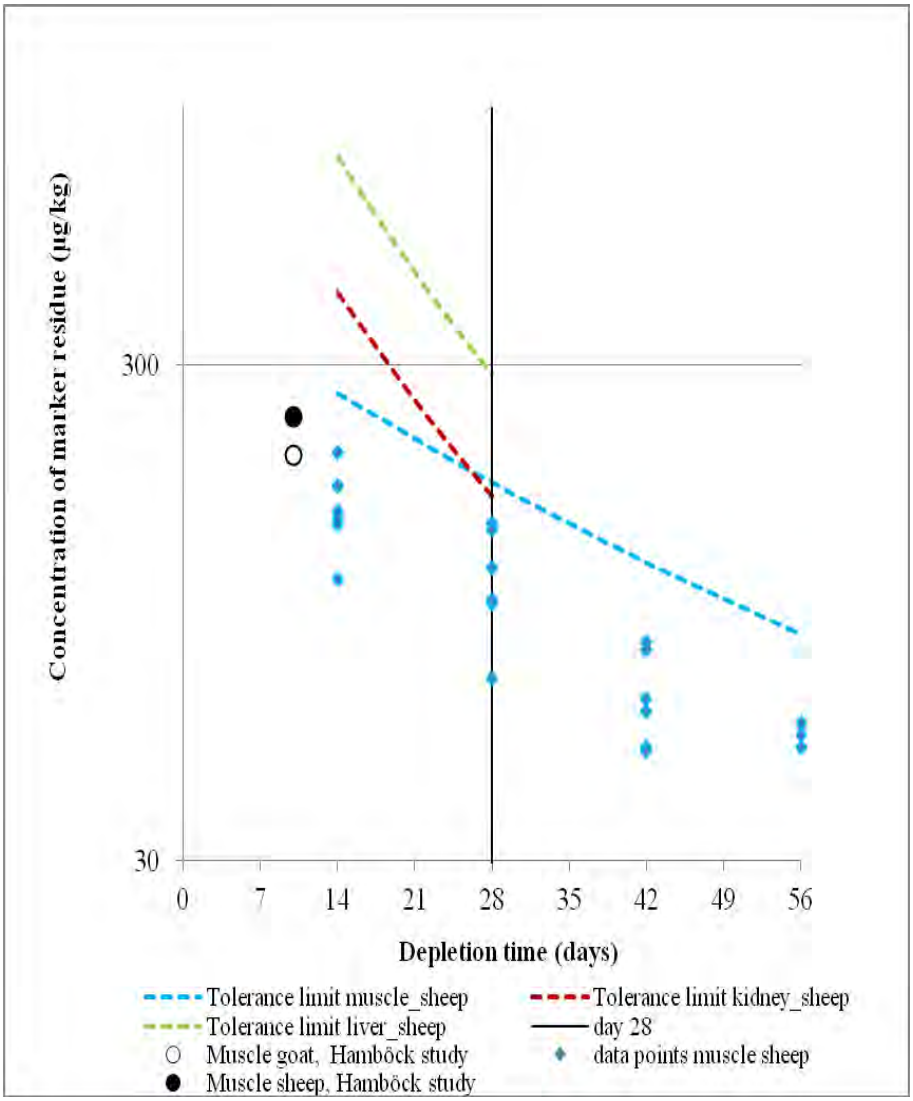

Figure 7.3. Selected data and calculated results of the study in comparison with data points on marker residue determination in muscle of a single goat and of a single sheep

ESTIMATION OF DAILY INTAKE

Sheep

The 70th meeting of the Committee estimated the daily intake on the basis of the residue concentrations found in tissues of sheep on day 28 after withdrawal of treatment. The median concentration of the marker residue used for the calculation was based on a statistical evaluation of 12 to 21 data points per tissue. The calculated daily total intake of total residue equivalents of triclabendazole was 165 µg per person. This corresponds to approximately 92% of the ADI. This would be reduced to 21.5 µg per person (less than 12% of the ADI) when the bio-availability of the residues (approximately 13%) is taken into account.

Goat

A theoretical estimate of consumption of a standard food basket calculated from the data of Table 7.5 would result in intakes of 316 µg/day (176% of the ADI) on day 10 after treatment; however, taking the limited bio-availability of the residues into account and using the factor of 0.13 developed by the 70th meeting of the Committee for tissues of cattle on day 28 after treatment (see FAO, 2009), this

would be reduced to 41 µg/day (equivalent to 23% of the upper bound of the ADI). This estimate is based on one data point per tissue obtained from a study with a radiolabelled, non-commercial product in one single animal. The animal was slaughtered 10 days after treatment.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

The 70th meeting of the Committee reviewed available analytical methods according to their performance characteristics and results obtained in validation studies. Three methods were discussed for which the validation data were acceptable (FAO, 2009).

Several additional methods have been published in the open literature since, of which two are relevant. Cai *et al.* (2010) reported a method for the simultaneous determination of triclabendazole and its metabolites (the sulphoxide, sulphone and keto-triclabendazole) in bovine and goat tissues. The determinative step is based on HPLC-MS/MS with a deuterated triclabendazole internal standard. Validation data are provided. Cheng *et al.* (2011) published a multi-analyte method for several benzimidazoles, including triclabendazole and its sulphoxide and sulphone metabolites in edible tissues. The determinative step is also based on HPLC-MS/MS with deuterated fenbendazole as the internal standard. Validation data for several tissues were provided.

APPRAISAL

The 19th Session of the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) asked the question: "Can MRLs for goat (tissues) be established by extrapolation considering data used for recommending MRLs for cattle and sheep (tissues)". CCRVDF also noted that "JECFA has established MRLs for sheep and cattle and extrapolation would be based on the data packages available to the 70th JECFA and a literature review to be provided by the United States of America."

The sponsor of the dossier for the 70th JECFA re-submitted that information, together with an addendum to the expert report of 17 August 2005. The author of the addendum carried out a literature search with the keyword "triclabendazole" covering the period from January 2004 to 27 October 2010. No other literature review was received. The Committee extended the literature search to cover the period until 25 October 2011.

The Committee specifically re-evaluated pharmacokinetic, metabolism and residue data from the 40th, 66th, and 70th meetings that were considered relevant for possibly providing an answer to the question raised by CCRVDF, provided that linking data were found enabling between-species extrapolations. Some kinetic studies performed in cattle were not re-evaluated because it was unlikely to extrapolate from the kinetic residue data obtained with this species to the goat. The 70th meeting of the Committee had already concluded "that the kinetic behaviour of triclabendazole was distinctly different in cattle and sheep and that there was no basis for establishing MRLs of identical numerical values for the two species". However, many products used in sheep are also recommended for use in goat and the recommended doses are typically the same (with few exceptions, more or less uniformly, oral doses of 10 mg/kg bw). Therefore, it was important to examine the possibility to extrapolate from kinetic residue data obtained in sheep to goat.

No state-of-the-art comparative pharmacokinetic study conducted in the same laboratory and using a commercial product or equivalent formulation in a sufficient number of animals of both species of animal was available.

The complex comparative study carried out with radiolabelled triclabendazole and one single animal of both species (approximately 30 years ago) was of limited value. The cumulative excretion pattern of the radioactivity was very similar in both animals in that study and the metabolites identified were the same. However, these are insufficient criteria to conclude that residue kinetics would also be the same or quantitatively similar. Only about 2.4% of the administered radioactivity was calculated to be present in blood and tissues of the goat. Kinetics in plasma of the radioactivity was qualitatively similar in the treated goat and the treated sheep; however, they were quantitatively different. Radio-

activity in most tissues was significantly different in the two animals, with higher concentrations found in the sheep. Whether this is a representative finding cannot be judged on the basis of a single treated animal. In the goat, the ratio of marker residue concentration to total residue concentration is only known for muscle of one animal.

Taking together all available data from all studies, two tissues of sheep and three tissues of goat are not covered by such a ratio, and therefore a full comparison between the two species cannot be made. All known figures are based on observations in one or two animals and the time points for which they are known are partly different for sheep and goat (28 and 10 days, respectively); variability and time trends are not known, except that for muscle the numerical values obtained for calves and sheep on day 28 in another study were similar to those obtained in sheep and goat on day 10 after treatment.

As the report of the 70th meeting has explained, the modelling of dietary intake of residues present in sheep tissues could only be conducted at day 28 after treatment, that being the only day when the ratio of the marker residue concentration to total residue concentration was known for two tissues. In the case of the goat, this ratio is only known for muscle and at day 10 after treatment.

The results of modelling performed by the 70th meeting have shown that the bio-availability of residues must be taken into account. The factor developed by the 70th meeting for incurred residues in liver of cattle (13%) would need to be re-applied for all tissues of the three ruminants in the absence of complete data for the other tissues and species.

For recommending MRLs for goat, the procedure adopted at the 66th meeting of the Committee could not be used, because of the absence of all necessary data except the ratio of marker to total residue concentrations in muscle of a single goat slaughtered on day 10 after treatment.

MAXIMUM RESIDUE LIMITS

The procedure for deriving MRLs adopted at the 66th meeting of the Committee could not be used, because the necessary data were not available. The Committee concluded that the available database on the residues of triclabendazole in goat did not allow a scientifically justifiable extrapolation of MRLs to this species of animal. The Committee recommended that the criteria described should be met and the corresponding data provided before triclabendazole is proposed for re-evaluation with the aim of obtaining MRLs based on extrapolations between species

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Annex 1
Summary of JECFA evaluations of veterinary drug residues from the 32nd meeting to the present

The following table summarises the veterinary drug evaluations conducted by JECFA at the 32nd (1987), 34th (1989), 36th (1990), 38th (1991), 40th (1992), 42nd (1994), 43rd (1994), 45th (1995), 48th (1997), 50th (1998), 52nd (1999), 54th (2000), 58th (2002), 60th (2003), 62nd (2004), 66th (2006) , 70th (2008) meetings and Meeting 2010 specifically for ractopamine in pig tissues. These meetings were devoted exclusively to the evaluation of veterinary drug residues in food. This table must be considered in context with the full reports of these meetings, published as WHO Technical Report Series.

Some notes regarding the table:

- The "ADI Status" column refers to the ADI and indicates whether an ADI was established as a full ADI or if the ADI is temporary (T).
- Where an MRL is temporary, it is indicated by "T".
- Where a compound has been evaluated more than once, the data given are for the most recent evaluation, including the 70th meeting of the Committee.
- Where the compound has been considered at more than one JECFA meeting, the date given is that of the last meeting.

Substance	ADI (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Abamectin	0–1 (JMPR 1995)	Full	47 (1996)	100 50	Liver, Fat Kidney	Cattle	Avermectin B_{1a}
Albendazole	0–50	Full	34 (1989)	100 5000	Muscle, Fat, Milk Liver, Kidney	Cattle, Sheep	MRLs analysed as 2-amino-benzimidazole, expressed as albendazole equivalents
Amoxicillin	0–0.7	Full	75 (2011)	50 4	Muscle, Liver, Kidney, Fat Milk	Cattle, Pig, Sheep Cattle, Sheep	Amoxicillin
Apramycin	0–30	Full	75 (2011)	5000	Kidney	Cattle, Chicken	Apramycin
Avilamycin	0–2000 (as avilamycin activity)	Full	70 (2008)	200 300	Muscle, Kidney, Skin/Fat Liver	Pig, Chicken, Turkey, Rabbit Pig, Chicken, Turkey, Rabbit	Dichloroisoeverninic acid (DIA), expressed as avilamycin equivalents
Azaperone	0–6	Full	52 (1999)	60 100	Muscle, Fat Liver, Kidney	Pig	Sum of azaperone and azaperol
Benzylpenicillin	<30 µg/person /day of the penicillin moiety	Full	36 (1990)	50 4	Muscle, Liver, Kidney Milk	All species	Benzylpenicillin
Bovine somatotropins	Not specified	Full	50 (1998)	Not specified	Muscle, Liver, Kidney, Fat, Milk	Cattle	
Carazolol	0–0.1	Full	52 (1999)	5 25	Muscle, Fat/Skin Liver, Kidney	Pig	Carazolol. The Committee noted that the concentration of carazolol at the injection site may exceed the ADI that is based on the acute pharmacological effect of carazolol
Carbadox	No ADI		60 (2003)	No MRL			The Committee decided that quinoxaline-2-carboxylic acid is not an appropriate marker residue
Ceftiofur	0–50	Full	48 (1997)	1000 2000 6000 2000 100	Muscle Liver Kidney Fat Milk	Cattle, Pig Cattle	Desfuroylceftiofur
Cefuroxime	No ADI		62 (2004)	No MRL			

Substance	ADI (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Chloramphenicol	No ADI		62 (2004)	No MRL			
Chlorpromazine	No ADI		38 (1991)	No MRL			
Chlortetracycline	0–30	Full	58 (2002)	200	Muscle	Cattle, Pig,	Parent drugs, either singly or in combination
Oxytetracycline	(Group ADI)			600	Liver	Sheep, Poultry	Oxytetracycline only
Tetracycline				1200	Kidney		
				400	Eggs	Poultry	
				100	Milk	Cattle, Sheep	
				200	Muscle	Fish	
				200	Muscle	Giant prawn	
Clenbuterol	0–0.004	Full	47 (1996)	0.2	Muscle, Fat	Cattle, Horse	Clenbuterol
				0.6	Liver, Kidney	Cattle	
				0.05	Milk		
Closantel	0–30	Full	40 (1992)	1000	Muscle, Liver	Cattle	Closantel
				3000	Kidney, Fat	Sheep	
				1500	Muscle, Liver,		
				5000	Kidney		
				2000	Fat		
Colistin	0–7	Full	66 (2006)	150	Muscle, Liver, Fat	Cattle, Sheep, Goat, Chicken, Turkey, Pig, Rabbit	Residue definition is the sum of Colistin A and colistin B. The MRL includes skin + fat where appropriate (chicken, turkey, pigs).
				200	Kidney		
				50	Milk	Cattle, Sheep	
				300	Eggs	Chicken	
Cyfluthrin	0–20	Full	48 (1997)	20	Muscle, Liver, Kidney	Cattle	Cyfluthrin
				200	Fat		
				40	Milk		
Cyhalothrin	0–5	Full	62 (2004)	20	Muscle, Kidney	Cattle, Sheep, Pig	Cyhalothrin
				400	Fat	Cattle,	
				20	Liver	Pig	
				50	Liver	Sheep	
				30	Milk	Cattle, Sheep	

Substance	ADI (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Cypermethrin α-Cypermethrin	0–20 (Group ADI)	Full	62 (2004)	50 1000 100	Muscle, Liver, Kidney Fat Milk	Cattle, Sheep Cattle, Sheep	Total of cypermethrin residues (resulting from the use of cypermethrin or α-cypermethrin as veterinary drugs)
Danofloxacin	0–20	Full	48 (1997)	200 400 100 100 50 200 100	Muscle Liver, Kidney Fat Muscle Liver Kidney Fat	Cattle, Chicken Pig	Danofloxacin For chicken fat/skin
Deltamethrin	0–10 (1982 JMPR)	Full	60 (2003)	30 50 500 30 30	Muscle Liver, Kidney Fat Milk Eggs	Cattle, Chicken, Sheep, Salmon Cattle, Sheep, Chicken Cattle Chicken	Deltamethrin
Derquantel	0–0.3	Full	75 (2011)	0.2 0.7 2	Muscle, Kidney Fat Liver	Sheep	Derquantel
Dexamethasone	0–0.015	Full	70 (2008)	1 2 0.3	Muscle, Kidney Liver Milk	Cattle, Pig, Horse Cattle, Pig, Horse Cattle	Dexamethasone
Diclazuril	0–30	Full	50 (1998)	500 3000 2000 1000	Muscle Liver Kidney Fat	Sheep, Rabbit, Poultry	Diclazuril Poultry skin + fat
Dicyclanil	0–7	Full	60 (2003)	150 125 200	Muscle Liver, Kidney Fat	Sheep	Dicyclanil
Dihydro-streptomycin Streptomycin	0–50 (Group ADI)	Full	58 (2002)	600 1000 200	Muscle, Liver, Fat Kidney Milk	Cattle, Pig, Chicken, Sheep Cattle, Sheep	Sum of dihydrostreptomycin and streptomycin
Dimetridazole	No ADI		34 (1989)	No MRL			

Substance	ADI (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Diminazene	0–100	Full	42 (1994)	500 12000 6000 150	Muscle Liver, Kidney Milk	Cattle	Diminazene
Doramectin	0–1	Full	62 (2004)	10 5 100 30 150 15	Muscle Muscle Liver Kidney Fat Milk	Cattle Pigs Cattle, Pigs Cattle, Pigs Cattle, Pigs Cattle	Doramectin
Enrofloxacin	0–2	Full	48 (1997)	No MRL			
Eprinomectin	0–10	Full	50 (1998)	100 2000 300 250 20	Muscle Liver Kidney Fat Milk	Cattle	Eprinomectin B_{1a}
Erythromycin	0–0.7	Full	66 (2006)	100 50	Muscle, Liver, Kidney, Fat/Skin Eggs	Chicken, Turkey Chicken	Erythromycin A
Estradiol-17β	0–0.05	Full	52 (1999)	Not specified	Muscle, Liver, Kidney, Fat	Cattle	
Febantel Fenbendazole Oxfendazole	0–7 (group ADI)	Full	50 (1998)	100 500 100	Muscle, Kidney, Fat Liver Milk	Cattle, Goat, Horses, Pig, Sheep Cattle, Sheep	Sum of febantel, fenbendazole and oxfendazole, expressed as oxfendazole sulphone equivalents
Fenbendazole (see Febantel)							
Fluazuron	0–40	Full	48 (1997)	200 500 7000	Muscle Liver, Kidney Fat	Cattle	Fluazuron

Substance	ADI (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Flubendazole	0–12	Full	40 (1992)	10	Muscle, Liver	Pig	Flubendazole
				200	Muscle	Poultry	
				500	Liver		
				400	Eggs		
Flumequine	0–30	Full	66 (2006)	500	Muscle	Cattle, Sheep, Pig, Chicken	Flumequine.
				1000	Fat	Trout	The MRLs are temporary for Black Tiger Shrimp and Shrimp. The MRLs for shrimp applies to all fresh water and marine shrimp.
				500	Liver	Black Tiger Shrimp	
				3000	Kidney	Shrimp	
				500	Muscle		
				500T	Muscle		
				500T	Muscle		
Furazolidone	No ADI		40 (1992)	No MRL			
Gentamicin	0–20	Full	50 (1998)	100	Muscle, Fat	Cattle, Pig	Gentamicin
				2000	Liver	Cattle	
				5000	Kidney		
				200	Milk		
Imidocarb	0–10	Full	60 (2003)	300	Muscle	Cattle	Imidocarb, free base
				1500	Liver		
				2000	Kidney		
				50	Fat, Milk		
Ipronidazole	No ADI		34 (1989)	No MRL			
Isometamidium	0–100	Full	40 (1992)	100	Muscle, Fat, Milk	Cattle	Isometamidium
				500	Liver		
				1000	Kidney		
Ivermectin	0–1	Full	58 (2002)	100	Liver	Cattle	Ivermectin B_{1a}
				40	Fat	Cattle	
				15	Liver	Pig, Sheep	
				20	Fat	Pig, Sheep	
				10	Milk	Cattle	

Substance	ADI (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Levamisole	0–6	Full	42 (1994)	10	Muscle, Kidney, Fat	Cattle, Sheep, Pig, Poultry	Levamisole
				100	Liver	Cattle, Sheep, Pig, Poultry	
Lincomycin	0–30	Full	62 (2004)	200	Muscle	Chicken, Pig	Lincomycin
				500	Liver	Chicken, Pig	A separate MRL of 300 µg/kg for skin with adhering fat for pigs was recommended in order to reflect the concentrations found in skin of pigs and this MRL was also extended skin/fat for chicken.
				1500	Kidney	Pig	
				500	Kidney	Chicken	
				100	Fat	Chicken, Pig	
				150	Milk	Cattle	
Melengestrol Acetate	0–0.03	Full	66 (2006)	1	Muscle	Cattle	Melengestrol acetate
				10	Liver		
				2	Kidney		
				18	Fat		
Metronidazole	No ADI		34 (1989)	No MRL			
Monensin	0–10	Full	70 (2008)	10	Muscle, Liver, Kidney	Chicken, Turkey, Quail	Monensin
			75 (2011)	10	Muscle, Kidney	Cattle, Sheep, Goat	Cattle liver MRL revised at 75 JECFA
				20	Liver	Sheep, Goat	
				100	Liver	Cattle	
				100	Fat	Cattle, Sheep, Goat, Chicken, Turkey, Quail, Cattle	
				2	Milk		
Monepantel	0–20	Full	75 (2011)	300	Muscle	Sheep	Monepantel sulphone
				3000	Liver		
				700	Kidney		
				5500	Fat		
Moxidectin	0–2	Full	50 (1998)	20	Muscle	Cattle, Deer	Moxidectin
				50	Muscle	Sheep	The Committee noted very high concentrations and great variation in the residue levels at the injection site in cattle over a 49-day period after dosing.
				100	Liver	Cattle, Deer, Sheep	
				50	Kidney	Cattle, Deer, Sheep	
				500	Fat	Cattle, Deer, Sheep	

Substance	ADI (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Narasin	0–5	Full	70 (2008)	15	Muscle, Kidney	Chicken, Pig	Narasin A
			75 (2011)	50	Liver, Fat	Chicken, Pig	Temporary MRLs for cattle, replaced with full MRLs in cattle tissue
				15	Muscle, Kidney	Cattle	
				50	Liver, Fat	Cattle	
Neomycin	0–60	Full	60 (2003)	500	Muscle, Fat, Liver	Cattle, Chicken, Sheep, Turkey Goat, Pig, Duck	Neomycin
				10000	Kidney		
				500	Eggs	Cattle, Chicken, Sheep, Turkey Goat, Pig, Duck	
				1500	Milk	Chicken	
						Cattle	
Nicarbazin	0–400	Full	50 (1998)	200	Muscle, Liver, Kidney, Fat/Skin	Chicken (broilers)	N,N'-bis(4-nitrophenyl)urea
Nitrofurazone/ Nitrofural	No ADI		40 (1992)	No MRL			
Olaquindox	No ADI		42 (1994)	No MRL			The Committee recommended no MRLs but noted that 4µg/kg in muscle of pigs of the metabolite MQCA (3-Methylquinoxaline-2-carboxylic acid) is consistent with Good Veterinary Practice.
Oxfendazole (See Febantel)							
Oxolinic acid	No ADI		43 (1994)	No MRL			
Oxytetracycline (See chlortetracycline)							
Permethrin	No ADI		54 (2000)	No MRL			
Phoxim	0–4	Full	62 (2004)	50	Muscle, Liver, Kidney	Goat, Pig, Sheep	Phoxim
				400	Fat		
Pirlimycin	0–8	Full	62 (2004)	100	Muscle, Fat	Cattle	Pirlimycin
				1000	Liver		
				400	Kidney		
				100	Milk		

Substance	ADI (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Porcine Somatotropin	Not Specified		52 (1999)	Not Specified	Muscle, Liver, Kidney, Fat	Pig	
Procaine benzylpenicillin	< 30µg/person/ day of the penicillin moiety	Full	50 (1998)	50 4	Muscle, Liver, Kidney Milk	All species	Benzylpenicillin
Progesterone	0–30	Full	52 (1999)	Not Specified	Muscle, Liver, Kidney, Fat	Cattle	
Propionyl-promazine	No ADI		38 (1991)	No MRL			
Ractopamine	0–1	Full	66 (2006)	10 40 90	Muscle, Fat Liver Kidney	Cattle, Pig	Ractopamine
Ronidazole	No ADI		42 (1994)	No MRL			
Sarafloxacin	0–0.3	Full	50 (1998)	10 80 20	Muscle Liver, Kidney Fat/skin	Chicken, Turkey	Sarafloxacin
Spectinomycin	0–40	Full	50 (1998)	500 2000 5000 2000 200	Muscle Liver, Fat Kidney Eggs Milk	Cattle, Chicken, Pig, Sheep Chicken Cattle	Spectinomycin
Spiramycin	0–50	Full	48 (1997)	200 600 300 800 300 200	Muscle Liver Kidney Kidney Fat Milk	Cattle, Chicken, Pig Cattle, Chicken Pig Cattle, Chicken, Pig Cattle	For cattle and chicken, MRLs are expressed as the sum of spiramycin and neospiramycin. For pigs, the MRLs are expressed as spiramycin equivalents (antimicrobial active residues).
Streptomycin (See dihydro-streptomycin)							

Substance	ADI (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Sulfadimidine (Sulfamethazine)	0–50	Full	42 (1994)	100 25	Muscle, Liver, Kidney, Fat Milk	Cattle, Sheep, Pig, Poultry Cattle	Sulfadimidine
Sulfathiazole	No ADI		34 (1989)	No MRL			
Testosterone	0–2	Full	52 (1999)	Not specified	Muscle, Liver, Kidney, Fat	Cattle	
Tetracycline (See chlortetracycline)							
Thiamphenicol	0–5	Full	58 (2002)	No MRL			
Tiabendazole (Thiabendazole)	0–100	Full	58 (2002)	100 100	Muscle, Liver, Kidney, Fat Milk	Cattle, Pig, Goat, Sheep Cattle, Goat	Sum of tiabendazole + 5-hydroxy tiabendazole
Tilmicosin	0–40	Full	70 (2008)	100 1000 1500 300 1000 150 100 2400 1400 600 1200 250	Muscle, Fat Liver Liver Kidney Kidney Muscle Muscle Liver Liver Kidney Kidney Skin/Fat	Cattle, Pig, Sheep Cattle Sheep Pig Cattle, Sheep Pig Chicken Turkey Chicken Turkey Chicken Turkey Chicken, Turkey	Tilmicosin
Trenbolone acetate	0–0.02	Full	34 (1989)	2 10	Muscle Liver	Cattle	β Trenbolone for muscle α-Trenbolone for liver
Trichlorfon (Metrifonate)	0–2	Full	66(2006)	50 50	Milk Muscle, Liver, Kidney, Fat	Cattle	Trichlorfon Guidance MRLs at the limit of quantitation of the analytical method for monitoring purposes. No residues should be present in tissues when used with Good Veterinary Practice.

Substance	ADI (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Triclabendazole	0–3	Full	70 (2008)	250	Muscle	Cattle	Keto-triclabendazole
				850	Liver	Sheep	
				400	Kidney	Cattle, Sheep	
				200	Muscle		
				300	Liver		
				200	Kidney		
				100	Fat		
Tylosin	0–30	Full	70 (2008)	100	Muscle, Liver, Kidney	Cattle, Pig, Chicken	Tylosin A
				100	Fat	Cattle, Pig	
				100	Skin/Fat	Chicken	
				100	Milk	Cattle	
				300	Eggs	Chicken	
Xylazine	No ADI		47 (996)	No MRL			
Zeranol	0–0.5	Full	32 (1987)	2	Muscle	Cattle	Zeranol
				10	Liver		

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RESIDUE EVALUATION OF CERTAIN VETERINARY DRUGS

Joint FAO/WHO Expert Committee on Food Additives

75th meeting 2011

This document contains monographs on residue evaluations of certain veterinary drugs, prepared at the seventy-fifth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which was held in Rome , Italy from 8 to 17 November 2011. Four substances were evaluated for the first time for the animal species concerned - amoxicillin, apramycin, derquantel and monepantel. Three substances were reassessed, monensin, narasin and triclabendzole. Specifically, narasin was reassessed for an analytical method in cattle tissues only and triclabendazole for consideration only of extending the MRLs in sheep to goat tissues. The residue monographs provide information on chemical identity and properties of the compounds, pharmacokinetics and metabolism, residue depletion studies and analytical methods validated and used for the detection and quantification of the compounds. This publication and other documents produced by JECFA contain information that is useful to those who work with or are involved with recommending or controlling maximum residue limits for veterinary drugs in foods of animal origin.

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