Amoxicillin

First draft prepared by
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Identity

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

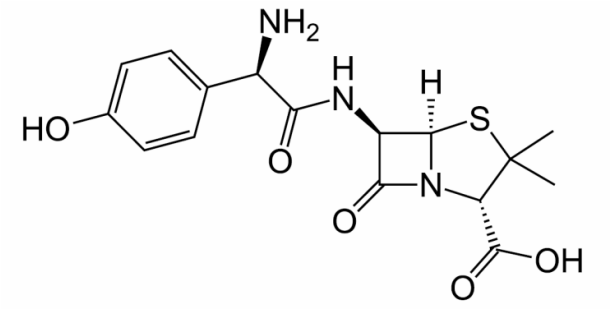
Synonyms: Amox; AMC; Amoxicillin trihydrate; Amoxicillin anhydrous; Amoxycillin trihydrate; D-Amoxicillin; p-Hydroxyampicillin

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.

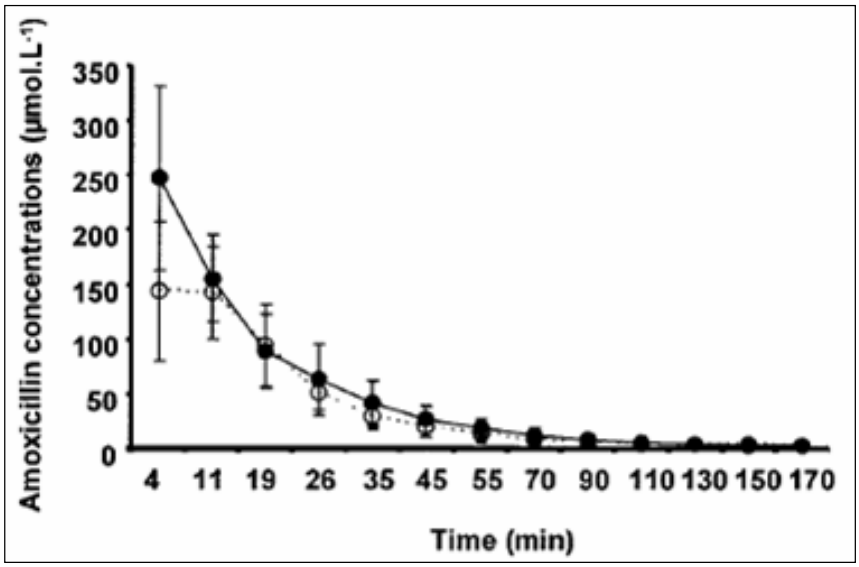


Figure 1.1. Unbound amoxicillin concentrations in blood and muscle of rats after intravenous (i.v.) bolus administration of amoxicillin at 50 mg/kg bw.
NOTES: Concentrations (mean ± SD) in blood (solid circles and solid line, n=11) and in muscle (open circles and dashed line, n=11)

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.

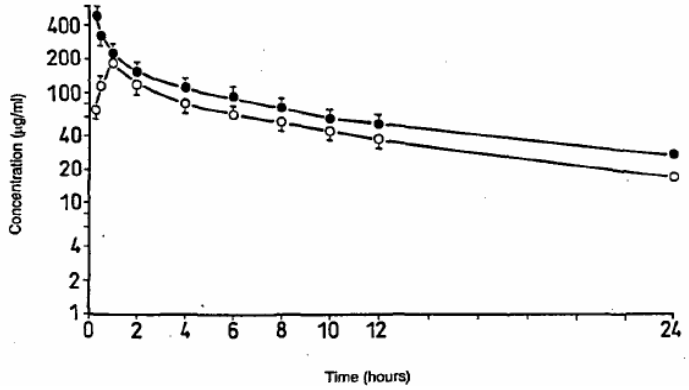


Figure 1.2. Plasma concentration of amoxicillin in chickens after intravenous (●) or oral (o) administration of 10mg/kg bw

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
$t_{½}α$ (h)	0.23 ± 0.01*	1.00 ± 0.10
$t_{½}β$ (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

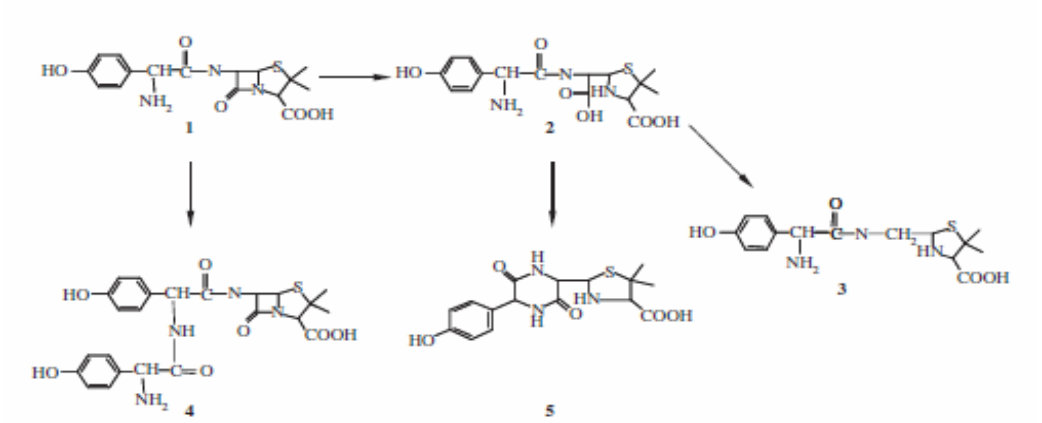


Figure 1.3. Principle metabolic pathway of amoxicillin.
KEY: (1) Amoxicillin; (2) Amoxicilloic acid; (3) Amoxilloic acid; (4) 4-Hydroxyphenylglycyl amoxicillin; (5) Amoxicillin piperazine-2',5'-dione.

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.

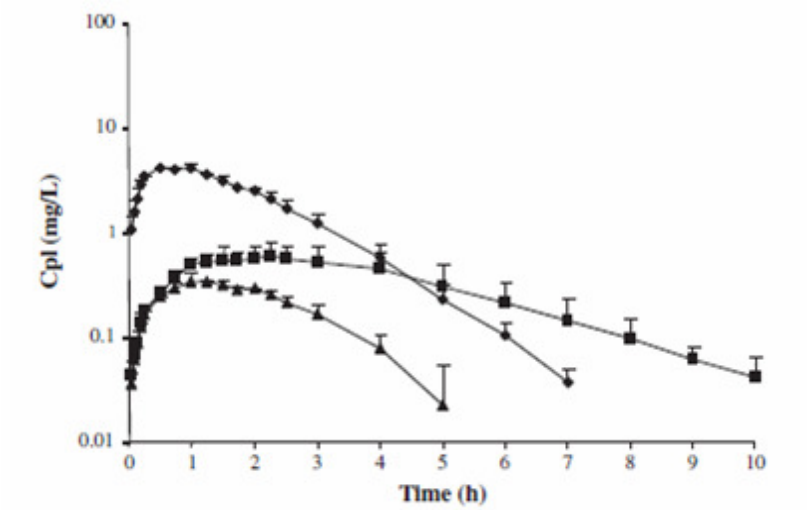


Figure 1.4. Plasma concentrations of amoxicillin, amoxicilloic acid and amoxicillin diketopiperazine in jugular venous plasma after single s.c. administration of amoxicillin at 20 mg/kg bw in pigs.
NOTES: Amoxicillin (diamond curve), amoxicilloic acid (square curve) and amoxicillin diketopiperazine (triangle 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	6.4	0.27	~1.2	<0.01	<0.01	0.18	<0.01
muscle	~4.5	0.19	<0.01	0.12	n.s.	n.s.	<0.01
	0.2	6.4	n.s.	~2.0	n.s.	<0.01	<0.01
Muscle	~0.40	~0.4	<0.01	<0.01	<0.01	<0.01	<0.01
	~0.40	<0.01	<0.01	<0.01	n.s.	n.s	<0.01
	0.31	<0.01	n.s.	<0.01	n.s.	<0.01	<0.01
Liver	~1.2	0.02	<0.01	<0.01	<0.01	<0.01	<0.01
	~1.2	0.01	<0.01	<0.01	n.s.	n.s	<0.01
	~1.2	0.02	n.s.	<0.01	n.s.	<0.01	<0.01
Kidney	~10	0.09	0.01	<0.01	<0.01	<0.01	<0.01
	~10	0.03	<0.01	0.02	n.s.	n.s	<0.01
	~10	0.05	n.s.	<0.01	n.s.	<0.01	<0.01
Fat	~0.4	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	0.2	0.02	<0.01	<0.01	n.s.	n.s	<0.01
	0.2	<0.01	n.s.	<0.01	n.s.	<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

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.

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.

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

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.

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

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

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.

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

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 1.25. 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

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

Tissue	Chemical	Time and route of administration							
		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.

An 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.
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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