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AMOXICILLIN page 3-37

Toxicological evaluation of certain veterinary drug residues in food

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AMOXICILLIN

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1. EXPLANATION

Amoxicillin (Chemical Abstracts Service [CAS] No. 26787-78-0) is a moderate-spectrum, semi-synthetic β-lactam antimicrobial agent used for treatment or prevention of bacterial infections in animals and humans. It exerts bactericidal effects by inhibiting the transpeptidase that catalyses the cross-linking of bacterial cell wall peptidoglycan. It is susceptible to degradation by β-lactamase and is often combined with clavulanic acid (a β-lactamase inhibitor) to improve its effectiveness against β-lactamase-producing bacteria.

In animals, amoxicillin is approved for use in cats, dogs, pigs, pre-ruminating calves, including veal calves, cattle, sheep, horses, chickens, ducks, pigeons and turkeys to treat a variety of Gram-positive (e.g. α- and β-haemolytic streptococci, *Streptococcus pneumoniae*, non-penicillinase-producing staphylococci and *Streptococcus faecalis*) and Gram-negative (e.g. many strains of *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Escherichia coli* and *Proteus mirabilis*) bacterial infections (all strains of *Pseudomonas* and most strains of staphylococci, *Klebsiella* and *Enterobacter* are resistant). Not all jurisdictions, however, have approved the drug in all species of animals listed above (USCFR, 2010; APVMA PUBCRIS, 2011; DPD, 2011; PID, 2011). Amoxicillin is stable in the presence of gastric acid and is a drug of choice within the class because of better oral bioavailability.

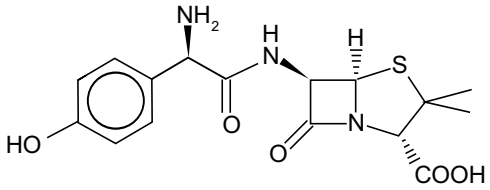
Amoxicillin, either as amoxicillin trihydrate (CAS No. 61336-70-7) or as sodium salt, has been used extensively in humans to treat a variety of infections (e.g. otitis media, pharyngitis and tonsillitis, lower respiratory tract infections, skin and skin structure infections, urinary tract infections, chlamydial infections, *Helicobacter pylori* infections and lyme disease). The World Health Organization (WHO) has categorized it as a critically important antimicrobial agent in human medicine.

In food-producing animals, amoxicillin is approved for use as amoxicillin trihydrate in oral suspensions equivalent to 40 mg of amoxicillin for baby pigs under 4.5 kg; as a soluble powder of amoxicillin trihydrate at 400 mg/45.5 kg body weight (bw) for pre-ruminating calves, including veal calves, administered by drench or by mixing in milk replacer; as amoxicillin trihydrate boluses containing 400 mg of amoxicillin for pre-ruminating calves; as a sterile amoxicillin trihydrate powder for use as a suspension, administered by intramuscular or subcutaneous injection in cattle; as a sterile intramuscular injection suspension containing amoxicillin at 50 mg/ml at a dose rate of 7 mg/kg bw for sheep; as a 150 mg/ml long-acting amoxicillin trihydrate oily intramuscular injection suspension for sheep; and as a 200 mg/ml intramuscular injection of amoxicillin for sheep, cattle and pigs.

Amoxicillin has not previously been reviewed by the Committee. The Committee carried out the present evaluation of amoxicillin to establish an acceptable daily intake (ADI) and to recommend maximum residue limits (MRLs) in cattle, sheep and pig tissues and cattle and sheep milk at the request of the Nineteenth Session of the Codex Committee on Residues of Veterinary Drugs in Foods (FAO/WHO, 2011).

Data supporting the evaluation of amoxicillin were provided by the United States Food and Drug Administration (USFDA) with the permission of the pharmaceutical sponsor. In addition, a literature search was conducted, which

Figure 1. Structure of amoxicillin



identified a number of additional pharmacokinetic and epidemiological studies that were used in this evaluation.

Amoxicillin, which has the International Union of Pure and Applied Chemistry name (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, has a hydroxyl group in the *para* position of the phenylglycine side-chain of ampicillin (Figure 1).

2. BIOLOGICAL DATA

2.1 Biochemical aspects

Studies conducted by the sponsor were performed prior to the implementation of good laboratory practice (GLP) guidelines, but consistent with the standards existing at the time of the study. Published journal articles reviewed often did not declare the GLP compliance status of their studies.

A few studies conducted by the sponsor on pharmacokinetics or toxicology identified the test article as BRL 2333 (the sponsor's code for amoxicillin) free acid or BRL 2333 sodium, but most identified it only as BRL 2333. The compound referred to as BRL 2333 is likely to be the amoxicillin trihydrate, but this could not be verified. This is, however, unlikely to have a significant impact on the interpretation of the studies, as the mass difference between amoxicillin and amoxicillin trihydrate is only 15%.

2.1.1 Absorption, distribution and excretion

(a) Laboratory animals

Amoxicillin administered orally to mice, rats and dogs was quickly absorbed. Peak plasma concentrations (C_{max}) above 5 µg/ml were observed within 1–2 hours post-administration (Table 1). Plasma concentrations were not proportional to the dose administered, suggesting a saturable absorption mechanism for amoxicillin. Amoxicillin distributed well into tissues and extravascular fluids. Protein binding in plasma, peripheral lymph and thoracic lymph in dogs was 15%, 9% and 7%, respectively (Acred et al., 1970). The drug was quickly eliminated from plasma, with an elimination half-life of 0.22 hour in mice (Woodnutt & Berry, 1999) and 1.5 hours in dogs (Küng & Wanner, 1994), with no accumulation.

Table 1. Pharmacokinetics of oral amoxicillin when administered to laboratory animals

Formulation	Species (strain, sex)	Dose (mg/kg bw)	C_{max} (μg/ml)	T_{max}	Reference
Free acid	Mouse (CS1, M)	50	11.2	10 min	Acred et al. (1970)
Trihydrate	Mouse (CS1/ OLAC, M/F)	25	9	10 min	Comber, Osborne & Sutherland (1975)
Trihydrate	Mouse (CS1, F?)	20	8.2	15 min	Woodnutt & Berry (1999)
Free acid	Rat (Sprague-Dawley, M)	100	33	30 min	Acred et al. (1970)
Free acid	Dog (Beagle, M/F)	100	15.1	2 h	Acred et al. (1970)
Free acid	Dog (Beagle, M/F)	100	35.9	1 h	Beecham Research Laboratories (1971a)
		1000	53.7	1 h	
		2000	50.0	1 h	
Not specified	Dog (Beagle, M/F)	11	5.5	1 h	Beecham Research Laboratories (1976)
Trihydrate	Dog (Beagle, M/F)	20	18.1–20.7[a]	1.4–2.0 h[a]	Küng & Wanner (1994)

C_{max}, peak plasma concentration; F, female; M, male; T_{max}, time to C_{max}
[a] Three different commercial formulations, suspension, tablet and drops, were investigated.

Studies using [^{14}C]amoxicillin at 100 mg/kg bw suggested that about 35.5% of the total radioactivity administered orally to rats was excreted in urine (Fairclough, Filer & Smith, 1971; Filer, 1971) and more than half (50.2%) was excreted in faeces (Fairclough, Filer & Smith, 1971). Radiolabelled amoxicillin administered to dogs at an oral dose of 85 mg/kg bw resulted in a higher amount (54%) of total radioactivity in urine compared with rats. Only 40.2% of radioactivity was detected in faeces of dogs by day 7 post-administration (Filer & Smith, 1971). Urinary excretion values for non-radiolabelled drug reported in many studies were lower than those reported in the radiolabelled studies (Acred et al., 1970), most likely because the microbiological assay would not detect metabolites that are microbiologically inactive.

The reported bioavailability of amoxicillin in rats was 44% when administered orally (Filer, 1971) and 51% when administered into the duodenal lumen (Chesa-Jiménez et al., 1994). In dogs, oral bioavailability ranged from 54% (Filer & Smith, 1971) to 77% (Küng & Wanner, 1994). Studies conducted in rats suggested the lack of hepatic first-pass metabolism for amoxicillin and that the low bioavailability of the drug in this species is related to presystemic degradation of amoxicillin in the intestine (Beecham Research Laboratories, 1971b; Chesa-Jiménez et al., 1994).

(b) Humans

As in laboratory animals, the pharmacokinetics of amoxicillin after oral administration to humans was characterized by rapid absorption, distribution and

Table 2. Pharmacokinetics of oral amoxicillin in humans

Formulation	Number of subjects	Dose (mg/ person)	C_{max} (µg/ml)	T_{max} (h)	Plasma elimination half-life (h)	Reference
Salt not specified	8	500	7.6	1.5	1.0	Gordon, Regamey & Kirby (1972)
Sodium salt	30	250	14.8	1.1	NR	Spyker et al. (1977)
		500	11.8	1.2	NR	
		1000	9.5	1.6	NR	
Trihydrate	9	500	9.5	1.5	1.0	Arancibia et al. (1980)
Trihydrate	16	500 (fasted)	8.9	1.9	8.9	Eshelman & Spyker (1978)
		500 (non-fasted)	8.8	2.4	8.8	
Trihydrate	12	375	8.1	1.0	1.7	Sjövall, Alván & Westerlund (1985)
		750	13.0	1.5	1.5	
		1500	17.9	1.75	1.6	
		3000	28.7	2.0	1.7	
Amoxicillin trihydrate–potassium clavulanate	144	250/125	4.3	1.1	1.4	Vree, Dammers & Exler (2003)
		500/125 tablet	6.8	1.5	1.5	
		500/125 suspension	7.6	1.1	1.5	
		875/125	9.9	2.0	1.8	

C_{max}, peak plasma concentration; NR, not reported; T_{max}, time to C_{max}

elimination, with no accumulation. The C_{max} for a single 500 mg/person dose of amoxicillin was between 5 and 10 µg/ml, with a time to C_{max} (T_{max}) of 1–2 hours (Table 2). The oral bioavailability was more than 80% (Spyker et al., 1977) and was not affected by the presence of food in the stomach (Eshelman & Spyker, 1978). Similar to laboratory animals, amoxicillin absorption in humans has a saturable mechanism, probably by a carrier-mediated transport (Spyker et al., 1977; Sjövall, Alván & Westerlund, 1985). The body clearance ranged from 0.2 l/kg bw per hour (Arancibia et al., 1980) to 0.34 l/kg bw per hour (Spyker et al., 1977). Within the normal dose range, amoxicillin pharmacokinetics was not affected by the presence of clavulanic acid (Vree, Dammers & Exler, 2003).

The urinary recovery of amoxicillin in humans as determined by a microbiological assay ranged from 43.4% to greater than 60% of the total dose (Beecham Research Laboratories, 1969b; Gordon, Regamey & Kirby, 1972; Spyker

Table 3. Amoxicillin recovery in 0- to 6-hour urine after oral administration to humans, rats and dogs

Subject (route)	% of dose recovered as amoxicillin	% of dose recovered as AMA	% of dose recovered as amoxicillin + AMA	% of amoxicilloic acid in total amoxicillin + AMA recovered in urine
Human (oral)	60	14	74	19
Rat (oral)	8	2	10	20
Rat (intramuscular)	62	10	72	14
Dog (oral)	11	7	18	39
Dog (intramuscular)	61	5	66	8

From Beecham Research Laboratories (1969b, 1970b)
AMA, amoxicilloic acid

et al., 1977; Arancibia et al., 1980). It is, however, realized that the microbiological methods would not detect biologically inactive metabolites. Studies conducted by the sponsor have demonstrated that metabolites constitute almost 20% of the total drug excreted in urine of rats, dogs and humans (Table 3). Renal excretion of amoxicillin occurs through both glomerular filtration and active tubular secretion (Sjövall, Westerlund & Alván, 1985).

2.1.2 Biotransformation

Most absorbed amoxicillin is excreted unchanged in the urine (Table 3). An in vitro incubation (with shaking) of amoxicillin at room temperature in hydrochloric acid solution (0.1 mol/l) for 1 hour identified amoxicilloic acid (AMA), amoxilloic acid, 4-hydroxyphenylglycyl amoxicillin and amoxicillin diketopiperazine-2′,5′-dione (DIKETO) as the degradation products (Nägele & Moritz, 2005). However, whether all of these metabolites are formed in vivo is not known. High-performance liquid chromatographic analysis has identified three metabolites: (5*R*,6*R*)-AMA, the (5*S*,6*R*) epimer and amoxicillin-(2*R*)-piperazine-2′,5′-dione in humans and pigs (Haginaka & Wakai, 1987; Reyns et al., 2008).

The pharmacokinetics of the major metabolites of amoxicillin (i.e. AMA and DIKETO) were compared in portal and jugular venous plasma after oral and intravenous administration to pigs (Reyns et al., 2009). Almost immediately after intravenous administration, high AMA and DIKETO concentrations were measured in plasma, whereas following oral dosing, the metabolites appeared in plasma after almost complete absorption of amoxicillin. No significant differences in pharmacokinetic parameters of amoxicillin, AMA and DIKETO, derived from the concentration–time profiles in portal and jugular venous plasma, were identified after intravenous or oral administration ($P > 0.05$). After oral administration, the half-life of elimination was 0.75 hour for amoxicillin and 2.7 hours for AMA, indicating a slower clearance of the metabolite. It is hypothesized that, compared with amoxicillin, which is excreted by both glomerular filtration and active secretion, AMA is eliminated only by glomerular filtration, as its open β-lactam structure might not be recognized by the transport carrier in the proximal tubule of the kidney.

Table 4. Results of studies of the acute toxicity of amoxicillin

Species	Sex	Route	LD_{50} (mg/kg bw)	Reference
Mouse	Male	Oral	>5000	Beecham Research Laboratories (1969a)
Rat	Male	Oral	>5000	Beecham Research Laboratories (1969a)
Rat	Male/female	Oral	>5500/8200	Hardy, Palmer & Cozens (1971)
Dog	Female	Oral	>20 000	Noel et al. (1970b)

LD_{50}, median lethal dose

Various studies suggest that only the parent compound has microbiological activity (Beecham Research Laboratories, 1969b, 1970b). However, both parent and some metabolites (e.g. AMA) could be potential allergens.

2.2 Toxicological studies

2.2.1 Acute toxicity

Results of acute toxicity studies with amoxicillin are summarized in Table 4.

(a) Mice

Groups of non-fasted male mice (10 per group) of the Ash CS1 strain, weighing 20–25 g, were administered amoxicillin by gavage at doses up to 5000 mg/kg bw. No deaths occurred and no clinical signs of toxicity were observed during the 5-day observation period (Beecham Research Laboratories, 1969a). The acute oral median lethal dose (LD_{50}) in mice was greater than 5000 mg/kg bw.

(b) Rats

Groups of non-fasted male Sprague-Dawley rats (six per group), weighing 140–160 g, were administered amoxicillin by gavage at doses up to 5000 mg/kg bw. No deaths occurred and no clinical signs of toxicity were observed during the 5-day observation period (Beecham Research Laboratories, 1969a). The oral LD_{50} in rats was greater than 5000 mg/kg bw.

Groups of CFY strain rats of both sexes, aged 3, 22 and 36 days, were administered a single dose of amoxicillin by gavage. The 3- and 22-day-old rats were treated at doses up to 5.5 g/kg bw. Among the 3-day-old male rats, five deaths occurred, one in each dose group, which were not treatment related. The 36-day-old male rats were treated at doses up to 5.5 g/kg bw, and females at doses up to 8.2 g/kg bw. No clinical signs of toxicity were observed during the 14-day observation period or at necropsy at the end of the observation period (Hardy, Palmer & Cozens, 1971). The oral LD_{50} was in excess of 5.5 g/kg bw.

(c) Dogs

Three female Beagle dogs, weighing 8.9–10.3 kg, were administered through a stomach tube single doses of amoxicillin at 10, 15 and 20 g/kg bw, with

Table 5. Results of short-term studies of the toxicity of amoxicillin

Species (sex)	Route	Doses	Treatment	NO(A)EL	Reference
Rat (M/F)	Oral	500 mg/kg bw per day	21 days	500 mg/kg bw per day (NOAEL)	Wheldon et al. (1968)
Rat (M/F)	Oral	500 mg/kg bw per day	21 days	500 mg/kg bw per day (NOAEL)	Beecham Research Laboratories (1970a)
Rat (M/F)	Oral	200, 500, 2000 mg/kg bw per day	26 weeks	2000 mg/kg bw per day (NOAEL)	Wheldon et al. (1970)
Cat (M/F)	Oral	100, 300, 500 mg/day	28 days	500 mg/day (NOAEL)	Ridglan Farms (1975)
Dog (M/F)	Oral	250 mg/kg bw per day	14 days	250 mg/kg bw per day (NOEL)	Noel et al. (1967)
Dog (M/F)	Oral	200, 500, 2000 mg/kg bw per day	6 months	2000 mg/kg bw per day (NOAEL)	Noel et al. (1970a)

F, female; M, male; NOAEL, no-observed-adverse-effect level; NOEL, no-observed-effect level

a 7-day gap between each dose. Some vomiting occurred 1–3 hours after dosing. No other clinical signs or adverse effects were seen during the 7-day observation period or at necropsy at the end of the study (Noel et al., 1970b). The LD_{50} in Beagle dogs was greater than 20 g/kg bw.

2.2.2 Short-term studies of toxicity

Results of short-term studies of the toxicity of amoxicillin are summarized in Table 5.

(a) Rats

Amoxicillin was administered by gavage to Sprague-Dawley rats (10 of each sex) at a dose of 500 mg/kg bw per day for 21 days and compared with vehicle-only controls. At the end of the study, all rats were necropsied. There were no mortalities and no marked effects on feed consumption, body weight or body weight gain. There were isolated intergroup differences in haematology and urine analysis parameters, which were considered not treatment related. The mean serum calcium level in treated females, but not males, was significantly lower, and albumin concentration was marginally elevated in treated females. The absolute and relative liver weights were lower in treated males ($P < 0.001$) and females ($P < 0.05$). No histological abnormalities were observed. There was no evidence of any changes in morphology that could be related to treatment (Wheldon et al., 1968). The no-observed-adverse-effect level (NOAEL) for this study was 500 mg/kg bw per day, the only dose tested.

In another 21-day study, groups of 10 male (132–139 g) and 10 female (123–126 g) Sprague-Dawley rats were administered amoxicillin in a 1% methylcellulose vehicle by gavage at a dose of 500 mg/kg bw per day. Control groups received the vehicle only. With the exception of three deaths (group not specified) due to errors of dosing (no information available on type of error), all other animals remained in good condition throughout the study. No treatment-related changes were observed in body weight gain, feed intake, haematology, blood chemistry or urine analysis. Histopathology revealed a minimal degree of statistically non-significant fatty change in the liver of treated females (Beecham Research Laboratories, 1970a). The NOAEL for this study was 500 mg/kg bw per day, the only dose tested.

Groups of Sprague-Dawley rats (25 animals of each sex per group) with mean body weights of 136 g (males) and 124 g (females) were administered amoxicillin by gavage at a dose of 0, 200, 500 or 2000 mg/kg bw per day, 6 days/week, for 26 weeks. At 13 weeks, 10 males and 10 females from each group were killed for interim study. On completion of treatment at 26 weeks, all remaining rats were necropsied. Moderate enlargement of caecum in male and female rats in the 2000 mg/kg bw per day group was the only treatment-related change observed at the interim necropsy. Absolute and relative liver weights were significantly higher in treated females of all groups, but relative liver weights in males were decreased in the high- and intermediate-dose groups. The relative spleen weight was depressed in females in the 2000 mg/kg bw per day treatment group. There were no dose-related changes in histology or in glycogen distribution. Therefore, changes in liver and kidney weights were considered not treatment related. At the terminal necropsy, there were no apparent changes in the absolute organ weights. Relative liver weight was marginally elevated in males, but not females, of the high-dose group. No treatment-associated changes were observed in hepatic structure or in glycogen distribution. No treatment-related changes were observed in blood biochemistry, haematology or urine analysis (Wheldon et al., 1970). The NOAEL for this study was 2000 mg/kg bw per day, the highest dose tested.

(b) Cats

Groups of cats (breeds not specified, two of each sex per group) were treated with amoxicillin trihydrate film–coated tablets at 0, 100, 300 or 500 mg/day, 5 days/week, for 4 weeks. The only lesion observed in the highest-dose males, but not females, was an increase in the level of renal tubular lipid. In the absence of related lesions in females, this effect was not considered of toxicological significance. No other treatment-related changes were identified (Ridglan Farms, 1975). The NOAEL for this study was the highest dose of 500 mg/animal, equal to 149 mg/kg bw per day, based on the group mean animal body weight of 3.36 kg at the initiation of the study.

In a second study, young cats (six of each sex per group) were treated with amoxicillin trihydrate at 11, 33 or 55 mg/kg bw per day by either the intramuscular or the subcutaneous route for 15 days. There were no effects on clinical signs, survival or blood parameters (Wren, 1977).

(c) Dogs

One male and one female Beagle dog received amoxicillin orally in gelatine capsules at a dose of 250 mg/kg bw per day for 14 days. Gross pathological and histopathological examination of major organs at the end of the study did not reveal any significant changes. There were no abnormalities that could be considered related to drug treatment (Noel et al., 1967). The no-observed-effect level (NOEL) for this study was 250 mg/kg bw per day, the only dose tested.

Amoxicillin was administered orally to groups of Beagle dogs (six dogs of each sex per group) 7 days/week for 6 months. Animals were dosed at 200, 500 or 2000 mg/kg bw per day in gelatine capsules. A control group was left untreated (no placebo administration). At the end of 3 months of dosing, three males and three females from each group were killed for interim study. On completion of 6 months of dosing, the remaining three females and three males from each group were necropsied. There were no deaths during the dosing period. Some vomiting did occur either immediately after dosing or 1–4 hours after dosing in the highest-dose group. Grey-coloured faeces were observed mainly in the first 3 weeks in a dose-related manner in dogs receiving 500 or 2000 mg/kg bw per day. Weight gain in the highest-dose group was decreased compared with control dogs ($P < 0.01$ between 0 and 13 weeks and $P < 0.05$ between 13 and 26 weeks). At the interim necropsy, relative kidney weights were increased in the high- and intermediate-dose groups, but no treatment-related microscopic abnormalities were found. At the completion of the study after 6 months, relative liver weights were increased in the highest-dose group. Microscopic examination revealed no changes in morphology associated with drug administration (Noel et al., 1970a). The decreased weight gain observed in the highest-dose group was likely associated with the disturbance in the gastrointestinal flora from the microbiological effect. The microbiological effect of amoxicillin is evaluated separately in section 2.2.6. The NOAEL for this study was the highest treatment level of 2000 mg/kg bw per day.

2.2.3 Long-term studies of toxicity and carcinogenicity

Long-term animal studies to evaluate the carcinogenic potential of amoxicillin were not performed. Multiple searches of the published literature and the web sites of public and environmental health organizations (e.g. International Agency for Research on Cancer, United States National Institute of Environmental Health Sciences) did not indicate any studies or reports on the carcinogenic effects of amoxicillin.

2.2.4 Genotoxicity

In an in vitro comet assay, amoxicillin induced deoxyribonucleic acid (DNA) damage at a 5 mmol/l concentration in human peripheral blood lymphocytes as well as in *Helicobacter pylori*–infected and non-infected human gastric mucosal cells. The damage was more pronounced in *H. pylori* non-infected gastric mucosal cells than in the infected gastric mucosal cells or peripheral lymphocytes. Amoxicillin-induced DNA damage was repaired within 60 minutes. The drug did not induce DNA strand breaks in isolated pUC19 plasmid DNA. There is some indication

that amoxicillin-induced DNA damage is an indirect effect due to the formation of oxidative free radicals (Arabski et al., 2005).

In a modified comet assay, amoxicillin at 5 mmol/l induced DNA lesions in vitro in human AGS cells. Some DNA damage was also noted at doses as low as 1 mmol/l. Repair of the amoxicillin-induced DNA lesions was essentially completed within 4 hours. Amoxicillin induced intracellular reactive oxygen species at a pace similar to that of induction of DNA lesions, suggesting that DNA damage caused by amoxicillin is likely an indirect effect due to the formation of reactive oxygen species (Li et al., 2007). Earlier studies have indicated that other β-lactams can also cause DNA damage in vitro at high concentrations, again possibly through induction of reactive oxygen species (Quinlan & Gutteridge, 1988).

Another in vitro study investigated the potential genotoxic effects of amoxicillin at concentrations of 400, 600, 800 and 1000 µg/ml, using sister chromatid exchange, chromosomal aberration and micronucleus tests in human peripheral blood lymphocytes. Amoxicillin did not induce sister chromatid exchanges or chromosomal aberrations in human peripheral blood lymphocytes in both the presence and the absence of a metabolic activator. Amoxicillin did not induce the formation of micronuclei or decrease the nuclear division index in human peripheral blood lymphocytes in both the presence and the absence of a metabolic activator (Istifli & Topaktas, 2010).

The Center for Drug Evaluation and Research of the USFDA reviewed additional genotoxicity data while assessing the safety of a human drug consisting of a combination of amoxicillin and potassium clavulanate. In the studies reviewed by the USFDA, but not available to the Committee for review, the combination of amoxicillin–clavulanic acid gave negative results for genotoxicity in vitro in an Ames test, a human lymphocyte cytogenicity assay and a yeast recombination assay. Amoxicillin–clavulanic acid was weakly positive in a mouse lymphoma assay only at cytotoxic concentrations. It was negative in vivo in a mouse micronucleus test and a dominant lethal test (USFDA, 2006).

Results of genotoxicity tests with amoxicillin are summarized in Table 6.

2.2.5 Reproductive and developmental toxicity

Results of reproductive and developmental toxicity studies are summarized in Table 7.

(a) Reproductive toxicity

(i) Rats

Groups of Specific Pathogen Free rats of the CD strain (11 males and 22 females per group) were treated with amoxicillin at a dose of 0, 200 or 500 mg/kg bw per day by gavage. Drug administration commenced when the males were 42 days of age and the females were 91 days of age and continued throughout the remainder of the investigation (the length of the treatment period was not specified). The animals were 105 days of age at cohabitation. On day 13 of gestation,

Table 6. Results of genotoxicity tests with amoxicillin

Test system/end-point	Test object	Concentration/dose	Result	Reference
Comet nuclear extraction assay for DNA strand breaks	Human AGS cell line	1–10 mmol/l	Positive (maximum effect observed at 5 mmol/l)	Li et al. (2007)
	Human NB4 cell lines	5 mmol/l		
	Chinese hamster CHP-K1 and UV24 cell lines	5 mmol/l		
Comet nuclear extraction assay for DNA strand breaks	Human peripheral lymphocytes	1–5 mmol/l	Positive (at 5 mmol/l)	Arabski et al. (2005)
	H. pylori–infected human gastric mucosa	1–5 mmol/l	Positive (at 5 mmol/l)	
	H. pylori non-infected human gastric mucosa	1–5 mmol/l	Positive (at 5 mmol/l)	
	Isolated pUC19 plasmid DNA	1–5 mmol/l	Negative	
Sister chromatid exchange	Human peripheral blood lymphocytes with/without S9 mix	400–1000 µg/ml	Negative	Istifli & Topaktas (2010)
Chromosomal aberration			Negative	
Micronucleus test			Negative	

DNA, deoxyribonucleic acid; S9, 9000 × *g* rat liver supernatant

Table 7. Results of reproductive and developmental toxicity studies of amoxicillin in laboratory animals

Species (sex)	Dose (mg/kg bw per day)	Treatment schedule and study description	Outcome	Reference
Mouse (F)	0, 200, 500, 2000	Treated on days 6–15 and killed on day 17 of gestation	Equivocal results on fetal loss, NOAEL could not be identified	Palmer & Cozens (1969b)
Rat (F)	0, 200, 500, 2000	Treated on days 6–15 and killed on day 20 of gestation	NOEL for developmental toxicity 2000 mg/kg bw per day	Palmer & Cozens (1969a)
Rat (M/F)	0, 200, 500	Males treated from 42 days of age, females from 91 days of age, cohabited on day 105 and killed on day 13 of gestation (first mating) or allowed to rear pups, remated and killed on day 20 of gestation (second mating)	NOAEL for reproductive toxicity 500 mg/kg bw per day	Palmer & Cozens (1970)
Rat (F)	0, 200, 500	Treated from day 15 of gestation through lactation day 21	NOAEL for perinatal and postnatal developmental toxicity 500 mg/kg bw per day	Palmer & Cozens (1969c)

F, female; M, male; NOAEL, no-observed-adverse-effect level; NOEL, no-observed-effect level

10 females from each group were necropsied for examination of the reproductive tract and the fetuses. The remaining rats were allowed to rear their pups. They were mated again for a second time and killed on day 20 of gestation.

Parent males showed a treatment-related retardation of body weight gain. A similar trend was not apparent among females. Among rats necropsied at the first gestation, there was a reduction in the corpora lutea count in the 500 mg/kg bw per day group. This was not considered to be of toxicological significance, as a similar effect was not observed in the second mating, and the implantation rate and the number of viable pups in treated animals were not statistically different from those of controls. No abnormal pups were observed and no other differences were observed between the treated and control animals or their pups after both the first and second matings (Palmer & Cozens, 1970).

The NOAEL for reproductive toxicity was 500 mg/kg bw per day, the highest dose tested.

(b) Developmental toxicity

(i) Mice

Amoxicillin was administered to groups (20 per group) of Specific Pathogen Free CD-1 mice. Animals were dosed at 0, 200, 500 or 2000 mg/kg bw daily by gavage on days 6 through 15 of pregnancy. On day 17, animals were killed and the ovaries and uterine contents were examined for the number of resorption sites, number of viable pups and developmental abnormalities in pups.

Among the parent animals, body weight change and pregnancy rate were unaffected by treatment, and no deaths or other abnormalities were identified. Embryonic and fetal development as assessed by the incidence of major malformations, minor visceral anomalies and the distribution of skeletal variants was unaffected by treatment. Fetal losses of 4.8%, 13.9%, 15.2% and 13.0% were observed in the 0, 200, 500 and 2000 mg/kg bw per day dose groups, respectively. Although the difference in fetal loss between the control and treated groups was significant, these values were within the historical control range of 3.8–14.3% (mean 7.9%) for the laboratory. Additionally, there was no dose–response effect on fetal loss (Palmer & Cozens, 1969b).

The results of this study were considered equivocal, and a NOAEL for developmental toxicity in mice could not be identified.

(ii) Rats

Amoxicillin was administered to groups of Specific Pathogen Free rats (20 per group) of the CD strain at a dose of 0, 200, 500 or 2000 mg/kg bw daily by gavage on days 6 through 15 of pregnancy. On day 20, the animals were killed and the ovaries and uterine contents were examined for the numbers of corpora lutea, resorption sites, viable pups and abnormalities. There were no alterations in clinical signs, survival, pregnancy rate, preimplantation loss, litter size, litter weight, major malformations, minor visceral anomalies or the distribution of skeletal variants

in any treatment group. In the medium- and high-dose groups, there was a dose-related increase in mean pup weight (Palmer & Cozens, 1969a). The NOEL for developmental toxicity was the highest tested dose of 2000 mg/kg bw dose per day.

Amoxicillin was administered to groups of Specific Pathogen Free CD strain rats (20 per group) at a dose of 0, 200 or 500 mg/kg bw per day by gavage from day 15 of gestation through to day 21 of lactation. After parturition, all pups were counted, weighed and examined. There were no effects on clinical signs, body weight gain, pregnancy rate, duration of gestation, and pup weights or abnormalities at birth. Pups from treated groups had a better weight gain postpartum. The total litter size at birth was 11.5, 10.3 and 9.6 for the 0, 200 and 500 mg/kg bw treatment groups, respectively, which was dose dependent. However, there was no difference in the number of dead pups at birth (Palmer & Cozens, 1969c). The difference in the litter size was likely due to differences in the implantation rate and was not considered treatment related, as drug administration started post-implantation (i.e. day 15 of gestation). The NOAEL for perinatal and postnatal toxicity was 500 mg/kg bw per day, the highest dose tested.

The effects of amoxicillin on Sprague-Dawley rat kidney development were studied by in vitro metanephros organ cultures and in vivo maternal treatment. For the in vitro experiments, metanephroi were removed from 14-day-old fetuses and grown in cultures containing amoxicillin at a concentration of 0, 10, 100 or 1000 µg/ml for 2 days. For the in vivo experiments, pregnant rats were treated with amoxicillin from day 11 to day 15 of gestation at 100 mg/kg bw per day through a subcutaneous implant. Amoxicillin altered renal development in vitro in a dose-dependent manner at concentrations at or above 100 µg/ml. In pups exposed to amoxicillin in utero, no reduction in the number of nephrons per gram of body weight was detected, but kidney histology showed mild cystic tubule dilatation (Nathanson et al., 2000). The toxicological relevance of this study is not clear.

2.2.6 Special studies on microbiological effects

A JECFA decision-tree approach that was adopted by the sixty-sixth meeting of the Committee (Annex 1, reference *181*) and that complies with International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) Guideline 36 (GL36) was used by the Committee to determine the need to establish a microbiological ADI for amoxicillin (VICH, 2004). The decision-tree approach initially seeks to determine if there may be microbiologically active amoxicillin residues entering the human colon. If the answer is “no” to any of the first three steps, then no microbiological ADI is necessary. However, should such residues be present, then two end-points of public health concern are to be considered: 1) disruption of the colonization barrier and 2) increase of the population(s) of resistant bacteria. At Step 4 of the decision-tree process, it is possible to provide scientific justification to eliminate testing (i.e. the need for a microbiological ADI) for either one or both of these end-points. At Step 5, a microbiological ADI would be determined. Should a microbiological ADI not be necessary, then the toxicological or pharmacological ADI would be used.

The Committee evaluated in vitro minimum inhibitory concentration (MIC) susceptibility testing data and in vivo human volunteer, biological activity of

Table 8. MIC of amoxicillin against* Lactobacillus *and* Bifidobacterium *species

Strains	Total no. of strains	No. of strains with indicated MIC values			
		≤0.25 µg/ml	0.5 µg/ml	1 µg/ml	2 µg/ml
Bifidobacterium longum	47	35	10	1	1
B. bifidum	16	16	—	—	—
B. pseudocatenulatum	11	7	4	—	—
B. catenulatum	2	2	—	—	—
Lactobacillus gasseri	20	9	11	—	—
L. delbrueckii	8	8	—	—	—
L. casei/L. paracasei	7	—	5	2	—
L. rhamnosus	5	—	3	2	—
L. acidophilus	2	—	2	—	—
L. plantarum	1	—	1	—	—
L. parabuchneri	1	—	1	—	—
L. brevis	1	1	—	—	—
L. vaginalis	1	—	—	1	—

From Delgado, Flórez & Mayo (2005)

amoxicillin residue and antimicrobial resistance studies for use in the decision-tree to answer the following questions in the assessment of amoxicillin.

Step 1: Are residues of the drug, and/or its metabolites, microbiologically active against representatives of the human intestinal microflora?

Yes. Amoxicillin is microbiologically active against some bacterial genera and species representative of the human intestinal microflora. The MIC of amoxicillin was determined for 122 strains of *Bifidobacterium* and *Lactobacillus* species isolated from the faeces of eight healthy adult volunteers. Most of the bifidobacteria and lactobacilli strains assayed were susceptible to amoxicillin, with MIC values ranging from ≤0.25 to 0.5 µg/ml. Results are tabulated in Table 8 (Delgado, Flórez & Mayo, 2005). In an investigation of the antimicrobial susceptibility of 50 strains of bifidobacteria belonging to eight species, isolated from humans, animals or probiotic products, all 50 strains were sensitive to amoxicillin, with a minimum inhibitory concentration required to inhibit the growth of 50% of organisms (MIC_{50}) range of ≤0.06–0.25 µg/ml (Moubareck et al., 2005). In another study, the susceptibility of 492 anaerobic bacteria to 23 antimicrobial agents, including amoxicillin, was presented as per cent susceptible to indicate an amoxicillin concentration for each species. Amoxicillin was active against many of the strains tested at 8 µg/ml or less. The antibacterial spectrum is shown in Table 9 (Sutter & Finegold, 1976). The MIC_{50} values for the predominant and relevant intestinal microbiota were determined from

Table 9. Activity of amoxicillin against anaerobic bacteria

Bacteria	No. of strains	Cumulative % susceptible to indicated concentration (μg/ml)										
		≤0.1	0.5	1	2	4	8	16	32	64	128	≥256
Bacteroides fragilis[a]	42	2	5	10	17	26	31	52	83	91	93	100
Bacteroides melaninogenicus	59	71	83	86	90	95	100	—	—	—	—	—
Other *Bacteroides* and *Selenomonas* species	21	43	57	62	67	76	—	86	—	95	100	—
Fusobacterium nucleatum	8	63	88	—	100	—	—	—	—	—	—	—
Other *Fusobacterium* species	12	42	67	—	—	—	—	75		83	92	100
Peptococcus and *Gaffkya* species	19	42	90	95	100	—	—	—	—	—	—	—
Peptostreptococcus species	15	53	87	93	—	—	—	100	—	—	—	—
Anaerobic and microaerophilic streptococci	6	67	83	—	—	—	—	100	—	—	—	—
Gram-negative cocci	7	57	86	—	—	—	100	—	—	—	—	—
Eubacterium species	7	57	100	—	—	—	—	—	—	—	—	—
Arachnia propionica	2	—	100	—	—	—	—	—	—	—	—	—
Propionibacterium species	4	25	75	100	—	—	—	—	—	—	—	—
Actinomyces species	16	63	100	—	—	—	—	—	—	—	—	—
Lactobacillus species	10	60	90	—	100	—	—	—	—	—	—	—
Clostridium perfringens	8	75	88	—	—	—	—	—	100	—	—	—
Other *Clostridium* species	26	8	77	92	96	—	100	—	—	—	—	—

From Sutter & Finegold (1976)

[a] Includes all strains identified as subspecies of *B. fragilis.*

the antimicrobial susceptibility studies described for amoxicillin—i.e. *Escherichia coli* (MIC_{50} = 5 µg/ml), *Bifidobacterium* (MIC_{50} = 0.06 µg/ml), *Clostridium* (MIC_{50} = 0.1 µg/ml), *Bacteroides* (MIC_{50} = 0.5 µg/ml), *Lactobacillus* (MIC_{50} = 0.25 µg/ml), *Fusobacterium* (MIC_{50} = 0.1 µg/ml), *Eubacterium* (MIC_{50} = 0.1 µg/ml) and *Peptostreptococcus* (MIC_{50} = 0.1 µg/ml).

Several studies were available on the effects of orally administered amoxicillin (with and without clavulanic acid) on the intestinal microflora of humans. Ten healthy volunteers received 500 mg amoxicillin tablets every 8 hours for 7 days, and the impact on the oral and intestinal microflora was studied. Amoxicillin administration induced small alterations in the faecal microflora, although all subjects had overgrowth of new colonizing amoxicillin-resistant microorganisms, mainly *Escherichia coli*, *Klebsiella* and *Enterobacter*. β-Lactamase activity was detected in the flora of six volunteers (Brismar, Edlund & Nord, 1993). In another study, 12 healthy subjects (6 females, 6 males; ages 18–40 years) were given amoxicillin–clavulanic acid as 1000 mg tablets once daily for 7 days. Faecal samples were collected prior to, during and after administration (days –2 to 35) for microbiological analyses. The samples were diluted in pre-reduced media and inoculated aerobically and anaerobically on non-selective and selective media. Different colony types were identified to genus level by morphological, biochemical and molecular analyses. Amoxicillin–clavulanic acid administration caused increased numbers of enterococci and *Escherichia coli* in the aerobic intestinal microflora, whereas numbers of bifidobacteria, lactobacilli and clostridia decreased significantly. *Clostridium difficile* strains were recovered from three of the volunteers. The intestinal microflora of the volunteers returned to normal levels 35 days after the cessation of treatment (Lode et al., 2001).

Step 2: Do residues enter the human colon?

Yes. A number of residue studies using [^{14}C]amoxicillin were conducted to detect amoxicillin in food animal species (pigs, cattle, poultry, goats and sheep) and laboratory animals (rats, mice, monkeys, pigs, dogs and rabbits) by analytical chemistry (high-performance liquid chromatography–mass spectrometry) and microbiological assay methods. Muscle, liver, kidney, fat and skin from cattle and pig tissue and milk contain relatively low or no amoxicillin-derived residues, regardless of the period between withdrawal of medication and slaughter. Under the most conservative assumptions, amoxicillin residues may be present at low levels in meat products consumed by humans. Therefore, amoxicillin-related residues could enter the colon of a person ingesting edible tissues from treated animals.

Step 3: Do the residues entering the human colon remain microbiologically active?

Yes. As the submitted evaluation did not contain measurements of the amount of drug residue in the intestinal tract, the Committee used the pharmacokinetic and bioavailability studies to determine the fraction of oral dose available to the human intestinal microbiota. Pharmacokinetic and bioavailability studies indicated that amoxicillin is rapidly absorbed after oral administration. Human studies in healthy volunteers and patients gave a range of bioavailability figures for recovery of amoxicillin in urine. Amoxicillin is rapidly absorbed and is excreted in urine primarily in unchanged form. A considerable amount of the administered amoxicillin was

detected in faecal samples. Mastrandrea et al. (1984) reported that 50–60% of intact amoxicillin was detected in the urine 24 hours after dosing. Arancibia et al. (1980) showed that 43.4% and 57.4% of amoxicillin were recovered after oral and intravenous doses, respectively. Eshelman & Spyker (1978) showed 47% and 44% amoxicillin recovery in the urine of fasting and non-fasting children, respectively, after oral dosage. Beecham Research Laboratories (1969b) recovered 60% amoxicillin in the urine after an oral dose. Amoxicillin is metabolized to two major metabolites, AMA and DIKETO. Numerous studies indicate that only the parent drug amoxicillin has biological activity. However, no studies were available to evaluate whether metabolites detected in human urine are biologically active against bacteria. Therefore, amoxicillin residues entering the human colon will remain microbiologically active.

The lowest amoxicillin urinary recovery value from the human studies was 43.4%. Therefore, the fraction of oral dose available would be conservatively estimated as 56.6%.

Step 4: Is there any scientific justification to eliminate testing for either one or both end-points of concern, i.e. disruption of the colonization barrier or resistance development?

Yes. The Committee noted that there is scientific justification to eliminate resistance development as an end-point of concern. However, there is the potential for an adverse effect on the disruption of the colonization barrier in the human intestinal microbiota, as amoxicillin residues do occur in food animals in small amounts. Therefore, if edible tissues are ingested, they could have an impact on the intestinal microbiota. Furthermore, the susceptibility of bifidobacteria, a predominant microorganism in the gastrointestinal tract, to amoxicillin may affect the barrier effect, which prevents colonization of pathogenic bacteria in the gut (Moubareck et al., 2005). In terms of antimicrobial resistance, amoxicillin is a broad-spectrum β-lactam antibiotic used in both human and veterinary medicine. The widespread use of this antibiotic may give rise to resistant bacterial strains and reduce its efficacy in the treatment of disease. In a national and university hospital survey, high use of amoxicillin–clavulanic acid contributed to high rates of resistance in Enterobacteriaceae, including *E. coli*, up to 50%. Nationally, 8% of *E. coli* strains were resistant or intermediately susceptible to amoxicillin–clavulanic acid. Results of the influence of restriction on resistance from those antibiotics accounting for 90% of the university hospital antibiotic use were analysed for microbiological susceptibility of *E. coli* during two 9-month periods, before and after restricting the use of amoxicillin–clavulanic acid as well as other antibiotics. Using defined daily doses per 100 bed-days as the hospital measurement base, use of antibiotics including amoxicillin–clavulanic acid was 30% before intervention and declined to 4% after the intervention, and resistance of *E. coli* declined significantly from 37% to 11% (Matanovic et al., 2010). Although increased prevalence of resistance may be linked to amoxicillin used after therapeutic administration, amoxicillin residue levels in food-producing animals were either not detected or present at such low levels (below the MIC_{50} values of the representative human intestinal microbiota evaluated in this report) that it is unlikely that the development of resistance to amoxicillin would occur.

Step 5: Derivation of a microbiological ADI using the VICH GL36 approach

The formula for calculating the microbiological ADI from in vitro (MIC) data is as follows:

$$\text{Upper bound of the ADI (µg/kg bw)} = \frac{\text{MIC}_{calc} \times \text{Mass of colon content}}{\text{Fraction of oral dose available to microorganisms} \times \text{Body weight}}$$

The equation terms are derived as described below.

MIC_{calc}: In accordance with Appendix C of VICH GL36, calculation of the estimated no-observed-adverse-effect concentration (NOAEC) (MIC_{calc}) for colonization barrier disruption uses MIC values from the lower 90% confidence limit of the mean MIC_{50} for the most relevant and sensitive human colonic bacterial genera. The strains needed to determine the MIC_{calc} were chosen according to these guidelines, which state that an intrinsically resistant bacterial genus should not be included. Based on the MIC_{50} values for *Escherichia coli* (MIC_{50} = 5 µg/ml), *Bifidobacterium* (MIC_{50} = 0.06 µg/ml), *Clostridium* (MIC_{50} = 0.1 µg/ml), *Bacteroides* (MIC_{50} = 0.5 µg/ml), *Lactobacillus* (MIC_{50} = 0.25 µg/ml), *Fusobacterium* (MIC_{50} = 0.1 µg/ml), *Eubacterium* (MIC_{50} = 0.1 µg/ml) and *Peptostreptococcus* (MIC_{50} = 0.1 µg/ml), the MIC_{calc} is 0.10 µg/ml.

Mass of colon content: A value of 220 g is based on the colon content measured from humans.

Fraction of oral dose available to microorganisms: It is recommended that the fraction of an oral dose available for colonic microorganisms be based on in vivo measurements for the drug administered orally. Alternatively, if sufficient data are available, the fraction of the dose available for colonic microorganisms can be calculated as 1 minus the fraction (of an oral dose) excreted in urine. Human data are encouraged, but, in their absence, non-ruminant animal data are recommended. In the absence of data to the contrary, it should be assumed that metabolites have antimicrobial activity equal to that of the parent compound. The fraction may be lowered if the applicant provides quantitative in vitro or in vivo data to show that the drug is inactivated during transit through the intestine. Amoxicillin is rapidly absorbed and is excreted in urine primarily in unchanged form. The lowest amoxicillin urinary recovery data from the human studies was 43.4%. Therefore, the fraction of oral dose available would be 1 – 0.434 = 0.566.

Body weight: The body weight of an adult human is assumed to be 60 kg.

The upper bound of the microbiological ADI for amoxicillin is calculated as indicated below:

$$\text{Upper bound of ADI} = \frac{0.10 \text{ µg/ml} \times 220 \text{ g}}{0.566 \times 60 \text{ kg bw}}$$

$$= 0.65 \text{ µg/kg bw}$$

Therefore, a microbiological ADI of 0–0.7 µg/kg bw (rounded to one significant figure), or 42 µg for a 60 kg adult, was derived from in vitro MIC susceptibility testing.

2.3 Observations in humans

2.3.1 Gastrointestinal effects

Diarrhoea and vomiting are the most common adverse gastrointestinal effects reported in people treated orally with amoxicillin either alone or in combination with clavulanic acid. In a randomized trial in 354 children for treatment of otitis media, 164 children completed the study in the amoxicillin–clavulanic acid (90/6.4 mg/kg bw) treatment group. Among them, 17.9% had diarrhoea, 5.8% had vomiting, 6.4% had diaper rash and 1% had severe abdominal pain (Sher et al., 2005). Two systematic reviews of treatment of acute otitis media in children with amoxicillin and other antimicrobials (Takata et al., 2001; Coker et al., 2010) identified diarrhoea, rash and vomiting as reportable adverse effects related to treatment. Children receiving amoxicillin or ampicillin had a pooled diarrhoeal rate difference of 5% more than those receiving placebo, with rates as high as 14% in some studies (Coker et al., 2010). In another study, patients treated with amoxicillin–clavulanic acid had 18% more diarrhoea than did those treated with azithromycin (Takata et al., 2001). Other reviews of randomized clinical trials involving children (Marmor & Newman, 2011) reported an incidence of 20–33% diarrhoea in patients treated with amoxicillin–clavulanic acid. Diarrhoea occurred in 6% and 5% of breastfeeding infants when their mothers were treated with amoxicillin–clavulanic acid or amoxicillin, respectively (Benyamini et al., 2005).

2.3.2 Allergen and immune response

The cross-reactivity of amoxicillin to sera from benzylpenicillin-hypersensitive patients was demonstrated in vitro using a radioallergosorbent test or histamine release test (Anfosso, Leyris & Charpin, 1979). Several recent investigations in penicillin-sensitive humans have identified that amoxicillin is often the most frequently reactive penicillin tested (Blanca et al., 2001; Torres et al., 2003; Lin, Saxon & Riedl, 2010). Furthermore, about 80% of the patients that test positive to amoxicillin in the skin prick or intradermal tests are also positive to benzylpenicillin, and vice versa. There is also an indication that subjects who are positive to amoxicillin determinants become negative sooner than those positive to benzylpenicillin. In a prospective study, 60% of patients who were positive to benzylpenicilloyl or a minor determinant mixture (benzylpenicillin and benzylpenicilloic acid) remained skin test reactive after 5 years. However, all subjects who were positive to amoxicillin alone became skin test negative within the same period (Blanca et al., 1999).

Despite the widespread use of amoxicillin in food-producing animals for many years, hypersensitivity reactions associated with the consumption of amoxicillin residues in food were not identified in the literature search. However, oral exposure to a small quantity of amoxicillin in sensitized individuals was shown to elicit allergic reactions. For example, hypersensitivity was recorded in a mother who kissed her baby 3 hours after oral administration of amoxicillin to the baby

Table 10. Concentrations recommended for both prick and intradermal testing with β-lactams

Hapten	Dose
Benzylpenicilloyl	5×10^{-5} mmol/l
Minor determinant mixture	2×10^{-2} mmol/l
Amoxicillin	20–25 mg/ml (51.7 mmol/l)
Ampicillin	20–25 mg/ml (54 mmol/l)
Cephalosporin	1–2 mg/ml

Adapted from Torres et al. (2003)

(Mancuso & Berdondini, 2006) and in a woman who kissed her boyfriend a few minutes after he had taken oral amoxicillin (Pétavy-Catala, Machet & Vaillant, 2001). Hypersensitivity to amoxicillin has also been recorded in sensitized individuals from licking by a dog treated with amoxicillin (Tosoni et al., 2011), from various types of oral or contact exposure (Blanca et al., 1996; Kounis, Giannopoulos & Goudevenos, 2011), through sexual intercourse with a partner receiving amoxicillin or by drinking from a glass previously used for administering amoxicillin (Blanca et al., 1996).

Published literature comparing the allergenic potential of amoxicillin with that of other penicillins, including benzylpenicillin, could not be found. However, there is limited evidence that, compared with benzylpenicillin, amoxicillin is chemically less reactive with proteins to form haptens, both in vitro and in vivo. The concentrations used in routine β-lactam allergy testing to elicit hypersensitivity reactions may also be an indication of the relative allergenic potency of these compounds. Routine clinical allergic skin testing concentrations (expressed as millimoles per litre) of amoxicillin are typically 2500 times greater than those of benzylpenicillin (part of the minor determinant mixture) (Table 10).

The Committee previously assessed the toxicological information on benzylpenicillin (Annex 1, reference *91*) and procaine benzylpenicillin (Annex 1, reference *134*) and concluded that allergy was the determining factor in the safety evaluation of residues of benzylpenicillin. The thirty-sixth meeting of the Committee (Annex 1, reference *91*) recommended that the daily intake of benzylpenicillin from food be kept as low as practicable, and in any case below 30 µg of the parent drug. The risk associated with the occurrence of mild hypersensitivity reactions to benzylpenicillin at levels lower than this was considered to be insignificant. Given the cross-reactivity of amoxicillin and benzylpenicillin in a high proportion of penicillin-sensitive patients and the occurrence of hypersensitivity in sensitized individuals through oral exposure to a small amount of amoxicillin, the Committee considered the applicability of the previously established maximum allowable exposure limit of 30 µg of benzylpenicillin per person to amoxicillin.

2.3.3 Hepatotoxicity

Use of amoxicillin alone or in combination with clavulanic acid has been associated with liver injury in humans. Hepatotoxicity associated with the use of

amoxicillin alone is reported less frequently (e.g. Bolzan et al., 2000; Schwarze et al., 2002) than the hepatotoxicity associated with the use of amoxicillin in combination with clavulanic acid (Chang & Schiano, 2007; Robles et al., 2010; Andrade & Tulkens, 2011). Liver toxicity associated with amoxicillin–clavulanic acid is usually mild. It is generally observed 2 weeks after the initiation of treatment, and symptoms resolve within a few weeks (Andrade & Tulkens, 2011). There are, however, occasional reports of a protracted, more serious course of liver toxicity, including liver failure and death (Lucena et al., 2006). Prolonged or repeated course of treatment, heightened sensitivity in older (>65 years) males (Andrade & Tulkens, 2011) and certain genetic predisposition (O'Donohue et al., 2000) are considered risk factors for hepatotoxicity associated with amoxicillin–clavulanic acid.

The reported incidence of hepatotoxicity for amoxicillin in different studies ranges from less than 0.02 (Thiim & Friedman, 2003) to 3 (Garcia Rodriguez, Stricker & Zimmerman, 1997) per 100 000 prescriptions. In contrast, hepatotoxicity associated with amoxicillin–clavulanic acid has a much higher estimated incidence of 1–17 per 100 000 prescriptions (Larrey et al., 1992; Garcia Rodriguez, Stricker & Zimmerman, 1997; Hussaini et al., 2007). Hepatotoxicity is most frequently linked to the clavulanic acid moiety, as the use of the combination product increases the risk by about 9-fold compared with amoxicillin alone (Andrade & Tulkens, 2011). In epidemiological studies, the use of the amoxicillin–clavulanic acid combination was much more frequently associated with hepatotoxicity than the use of amoxicillin alone. Other studies have shown that in patients with a history of liver injury induced by amoxicillin–clavulanic acid, subsequent treatment with amoxicillin caused no hepatotoxicity, but treatment with amoxicillin and clavulanic acid led to a second episode of hepatitis (Stricker et al., 1989; Berg & Hahn, 2001). These observations indicate the predominant role of clavulanic acid in liver toxicity induced by the amoxicillin–clavulanic acid combination.

2.3.4 Reproductive toxicity

Several epidemiological studies evaluated the developmental toxicity or teratogenic potential of amoxicillin or amoxicillin–clavulanic acid used in pregnant women. In general, penicillins, including amoxicillin, are considered to have minimal reproductive toxicity. The potential reproductive toxicity of amoxicillin in humans was reviewed by Nahum, Uhl & Kennedy (2006). The results, summarized in Table 11, did not identify an increased risk of major congenital anomalies, malformations or teratogenic potential in children when their mothers were treated with amoxicillin during various stages of gestation. Based on these data, the increased risk of teratogenicity in humans is considered "unlikely".

An in vitro study investigated the effects of amoxicillin on sperm movement characteristics, viability and the ability of spermatozoa to undergo the acrosome reaction. Spermatozoa were cultured for 24 hours with increasing concentrations of antimicrobials. Amoxicillin had no effect on sperm movement characteristics over the dose ranges tested (2.5–500 µg/ml), although it inhibited viability at 1 mg/ml (44.7% in controls versus 38.4% in amoxicillin-treated groups). No effects were seen on the ability of spermatozoa to undergo the acrosome reaction (Hargreaves et al., 1998).

Table 11. Summary of the teratogenic and toxic potential of amoxicillin based on human and animal data

Human data: Teratogenic and toxic effects	Animal data: Teratogenic and toxic fetal effects
1. OR (adjusted) for congenital anomalies = 1.16 (95% CI 0.54–2.50) in a Danish study (1991–2000) of 401 primiparous women who filled prescriptions for amoxicillin during pregnancy (rate = 4.0%) compared with 10 237 controls who did not redeem any prescription drug (rate = 4.1%) (Jepsen et al., 2003). 2. No increased rate of congenital malformations among 147 women who received prescriptions for amoxicillin during the first trimester (Jepsen et al., 2003). 3. No increased rate of congenital anomalies among 284 infants whose mothers were administered amoxicillin or ampicillin during the first trimester or in 1060 infants whose mothers were treated at any time during pregnancy (Colley, Kay & Gibson, 1983). 4. No adverse effects in offspring exposed to amoxicillin during the second and third trimesters in three controlled clinical trials of antibiotic treatment for premature preterm rupture of membranes (Almeida, Schmauch & Bergstrom, 1996; Lovett et al., 1997; Mercer et al., 1997). 5. No significantly increased rate of major or minor anomalies in the children of 14 women treated with amoxicillin and probenecid during the first 14 weeks of gestation or among 57 women treated after the 14th week in a controlled clinical trial on the treatment of gonorrhoea during pregnancy (Cavenee et al., 1993). 6. OR for major congenital anomalies = 1.4 (95% CI 0.9–2.0) for women using amoxicillin–clavulanic acid during pregnancy in a case–control study of 6935 malformed infants (no increased risk) (Czeizel et al., 2001). 7. An association of necrotizing enterocolitis in newborns and maternal amoxicillin and clavulanic acid treatment during the third trimester was observed in a randomized controlled trial including 4826 pregnant patients (Kenyon et al., 2001; Kenyon, Taylor & Tarnow-Mordi, 2002).	1. No increased congenital malformations in mice treated with 3–7 times the maximum human therapeutic dose of amoxicillin (Abou-Tarboush, 1994). 2. Increased frequency of embryonic death in mice treated with amoxicillin at 6–7 times the maximum therapeutic human dose (Abou-Tarboush, 1994). 3. No adverse reproductive effects in rats given amoxicillin–clavulanic acid at doses of 400 and 1200 mg/day prior to fertilization and during the first 7 days of gestation (Hirakawa et al., 1983). 4. No adverse fetal effects in pigs given amoxicillin with clavulanic acid at a dose of 600 mg/kg bw on days 12–42 (James, Hardy & Koshima, 1983).

Modified from Nahum, Uhl & Kennedy (2006)
CI, confidence interval; OR, odds ratio

3. COMMENTS

Amoxicillin is an old drug with a long history of use. Studies were performed prior to the implementation of GLP guidelines, but consistent with standards existing at the time of study. Published journal articles reviewed often did not declare the GLP compliance status of their studies.

Data supporting the evaluation of amoxicillin were provided by the USFDA with the permission of the pharmaceutical sponsor. In addition, a literature search was conducted, which identified a number of additional pharmacokinetic and epidemiological studies that were used in this evaluation.

3.1 Biochemical data

Amoxicillin is rapidly absorbed through oral and parenteral routes of administration and distributed in various tissues. Peak plasma concentrations of amoxicillin greater than 10 µg/ml are observed generally within an hour after oral administration in mice (50 mg/kg bw), rats (100 mg/kg bw), dogs (100 mg/kg bw) and humans (500 mg/person). Oral bioavailability is approximately 50% in rats, about 65% in dogs and greater than 80% in humans. There is a dose-dependent saturability of amoxicillin absorption. Partial degradation of amoxicillin may occur in the intestine, thereby reducing its bioavailability. Plasma protein binding is not high (<15%). Amoxicillin has a short plasma elimination half-life (~1 hour), and the absorbed drug is quickly eliminated unchanged from the body, principally via urinary excretion through both glomerular filtration and active secretion. Hepatic first-pass metabolism is not observed. Within the normal dose range, amoxicillin pharmacokinetics is not influenced by the presence of clavulanic acid. Only the parent compound has microbiological activity.

3.2 Toxicological data

Acute toxicity results in mice, rats and dogs suggest that the drug is well tolerated at high doses following oral exposure (LD_{50} > 5000 mg/kg bw).

Short-term toxicity studies were conducted in rats, cats and dogs. In one study, rats of both sexes were administered amoxicillin by gavage at 500 mg/kg bw per day for 21 days. The absolute and relative liver weights in treated groups were significantly lower than those in the controls, but there were no histological changes that were treatment related. The NOAEL from this study was 500 mg/kg bw per day, the only dose tested.

In another study, male and female rats were treated with amoxicillin by gavage at 500 mg/kg bw per day for 21 days. Histopathological examination revealed minimal, statistically non-significant fatty changes in the livers of treated females. The NOAEL for this study was 500 mg/kg bw per day, the only dose tested.

Groups of male and female cats were treated orally with amoxicillin at 100, 300 or 500 mg/animal per day, 5 days/week, for 28 days. In the highest-dose males, there was an increase in the level of renal tubular lipid, but it was not considered to be treatment related. No other treatment-related lesions were identified. The NOAEL for this study was the highest dose tested, 500 mg/day per animal, equal

to 149 mg/kg bw per day based on the group mean animal body weight of 3.36 kg at the initiation of the study.

One male and one female dog were orally administered amoxicillin at a dose of 250 mg/kg bw per day for 14 days. Gross examination during necropsy and histopathological examination of major organs did not reveal any significant changes. The NOEL for this study was 250 mg/kg bw per day, the only dose tested.

In a 6-month study, male and female rats were dosed with amoxicillin by gavage at 0, 200, 500 or 2000 mg/kg bw per day, 6 days/week, for 26 weeks. At 13 weeks, some males and females from each group were necropsied; the remaining animals were necropsied at the end of the study. At the interim necropsy, moderate enlargement of the caecum in the highest-dose group was the only treatment-related change. The only change noticed at termination was elevated relative liver weights in the highest-dose males, but it was considered not to be of toxicological relevance. The NOAEL for this study was 2000 mg/kg bw per day, the highest dose tested.

In a 6-month study, male and female dogs were treated orally with amoxicillin at a dose of 200, 500 or 2000 mg/kg bw per day for 6 months. Control dogs were left untreated (no placebo administration). At the end of 3 months, half of the males and females from each group were necropsied; the remaining animals were necropsied at the end of 6 months. Some vomiting occurred either immediately or within 1–4 hours of dosing in some animals in the highest-dose group. Grey-coloured faeces were observed mainly in the first 3 weeks in a dose-related manner in dogs receiving 500 or 2000 mg/kg bw per day. The weight gain in the highest-dose group was decreased compared with controls. At termination of the study, relative liver weights were increased for the highest-dose dogs, but no associated histology was observed. The NOAEL for this study was 2000 mg/kg bw per day, the highest dose tested.

Amoxicillin was negative in vitro for sister chromatid exchange, chromosomal aberration and the induction of micronuclei in human peripheral lymphocytes with and without exogenous metabolic activation. In studies reviewed by the USFDA[1], but not available to the Committee, the combination of amoxicillin–clavulanic acid gave negative results for genotoxicity in vitro in an Ames test, a human lymphocyte cytogenicity assay and a yeast recombination assay. Amoxicillin–clavulanic acid was weakly positive in a mouse lymphoma assay only at cytotoxic concentrations. It was negative in vivo in a mouse micronucleus test and a dominant lethal test. As with other β-lactam antimicrobial agents, amoxicillin caused DNA damage in vitro in cultured cells at high concentrations. There is some evidence that this is an indirect effect due to the formation of reactive oxygen species.

The Committee concluded that it was unlikely that amoxicillin would be genotoxic in vivo following exposure to residues arising from its use as a veterinary drug.

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[1] Based on the USFDA's label on the approved human drug amoxicillin–clavulanate potassium.

Data from studies of long-term toxicity and carcinogenicity were not available for the Committee to review. However, the long history of use of amoxicillin in a wide range of species, including humans, has not identified an association between the use of amoxicillin and carcinogenicity. Similarly, there were no indications of potential carcinogenicity effects (e.g. increased incidence of tumours or preneoplastic lesions) in the 6-month rat study. These findings, together with the genotoxicity data reviewed, led the Committee to conclude that amoxicillin is unlikely to be carcinogenic to humans at levels of exposure to residues in food-producing animals.

In a reproductive toxicity study, rats were treated orally with amoxicillin at 200 or 500 mg/kg bw per day for several weeks before mating, during cohabitation and thereafter for the study duration, and animals were followed through first and second matings. No significant differences were noted between treated and control animals or their pups in most parameters measured after both the first and second matings, except reduced weight gain in treated males. The NOAEL from this reproductive study was 500 mg/kg bw per day, the highest dose tested.

Groups of pregnant mice were treated with amoxicillin at 0, 200, 500 or 2000 mg/kg bw per day by gavage between days 6 and 15 of pregnancy and killed on day 17. Fetal losses of 4.8%, 13.9%, 15.2% and 13.0%, respectively, were observed. No other treatment-related abnormalities were noted in either the dams or the pups. Although the difference in fetal losses between the control and treated groups was significant, these values were within the historical control range of 3.8–14.3% (mean 7.9%) for the laboratory, and there was no dose–response relationship. These results were considered equivocal, and a NOAEL for developmental toxicity in mice could not be identified.

Groups of pregnant rats were treated with amoxicillin at doses up to 2000 mg/kg bw per day by gavage between days 6 and 15 of pregnancy and killed on day 20. No significant effects were observed in either the dams or the pups from treated animals. The NOEL from this developmental toxicity study was 2000 mg/kg bw per day, the highest dose tested.

In another rat study, animals were treated with amoxicillin at 200 or 500 mg/kg bw per day by gavage from day 15 of gestation through to lactation day 21. There was no effect on dams, pup mortality or growth, and no abnormal pups were observed. The NOAEL for perinatal and postnatal toxicity was 500 mg/kg bw per day, the highest dose tested.

Epidemiological studies that investigated the risk of teratogenicity of amoxicillin in humans have not identified any increased risk. Based on the overall data reviewed, the Committee considered that amoxicillin is unlikely to cause reproductive or developmental toxicity in humans.

In humans, the use of amoxicillin–clavulanic acid has been reported to be associated with hepatotoxicity. However, hepatotoxicity is rarely associated with the use of amoxicillin alone. Information in the published literature suggests that clavulanic acid is likely to be responsible for this toxicity.

Hypersensitivity is considered to be the most relevant toxicological end-point for the safety assessment of amoxicillin. Individuals who are hypersensitive

to amoxicillin are also sensitive to other β-lactams, including benzylpenicillin. In recent studies, amoxicillin was often the most frequent positive allergen among several β-lactams tested in human patients, and a high proportion of patients who are reactive to amoxicillin (~80%) were also allergic to other penicillins. Although published literature indicates that hypersensitivity in sensitized individuals could be evoked by oral exposure to a small quantity of amoxicillin, no reports were found on hypersensitivity reactions occurring in humans from exposure to the residues of amoxicillin in foods of animal origin. The Committee previously evaluated benzylpenicillin and procaine benzylpenicillin, which dissociates to procaine and benzylpenicillin in the body (Annex 1, references *91* and *134*), and concluded that allergy was the determining factor in the safety evaluation of these residues, recommending that daily intake of benzylpenicillin from food be kept as low as practicable, and in any event below 30 µg of the parent drug.

Limited information available suggests that, compared with benzylpenicillin, amoxicillin is chemically less reactive with proteins to form haptens. Additionally, the recommended starting dilution of amoxicillin used in the diagnosis of hypersensitivity in sensitized individuals by the skin prick test is more than 2500 times higher than that of benzylpenicillin.

3.3 Microbiological data

A JECFA decision-tree approach that was adopted by the sixty-sixth meeting of the Committee (Annex 1, reference *181*) and that complies with VICH GL36 was used by the Committee to determine the need for, and to establish, if necessary, a microbiological ADI for amoxicillin. Studies of microbiological activity against bacterial strains representative of the human colonic flora were evaluated. Amoxicillin was active against *Escherichia coli* (MIC_{50} = 5 µg/ml), *Bifidobacterium* (MIC_{50} = 0.06 µg/ml), *Clostridium* (MIC_{50} = 0.1 µg/ml), *Bacteroides* (MIC_{50} = 0.5 µg/ml), *Lactobacillus* (MIC_{50} = 0.25 µg/ml), *Fusobacterium* (MIC_{50} = 0.1 µg/ml), *Eubacterium* (MIC_{50} = 0.1 µg/ml) and *Peptostreptococcus* (MIC_{50} = 0.1 µg/ml).

Amoxicillin residues may be present at low levels in meat products consumed by humans; therefore, amoxicillin-related residues could enter the colon of a person ingesting edible tissues from treated animals. Although amoxicillin was rapidly absorbed after oral administration in animals, a considerable amount of the administered amoxicillin was excreted through faeces. In the absence of data to support faecal inactivation, it is considered that amoxicillin residues entering the human colon will remain microbiologically active.

There is potential for disruption of the colonization barrier in the human gastrointestinal tract, as MIC values for the most relevant and predominant bacteria in the gastrointestinal tract indicated that they were susceptible to amoxicillin. Because the majority of amoxicillin residue levels detected in target tissue were below the lowest MIC_{50} of any of the representative human intestinal microbiota tested, it is unlikely that the development of resistance to amoxicillin residues would occur.

The formula for calculating the microbiological ADI is as follows:

$$\text{Upper bound of the ADI (µg/kg bw)} = \frac{MIC_{calc} \times \text{Mass of colon content}}{\text{Fraction of oral dose available to microorganisms} \times \text{Body weight}}$$

The equation terms are derived as described below.

MIC_{calc}: In accordance with Appendix C of VICH GL36, calculation of the estimated NOAEC (MIC_{calc}) for colonization barrier disruption uses MIC values from the lower 90% confidence limit of the mean MIC_{50} for the most relevant and sensitive human colonic bacterial genera. The strains needed to determine the MIC_{calc} were chosen according to these guidelines, which state that an intrinsically resistant bacterial genus should not be included. Based on the MIC_{50} values for *Escherichia coli* (MIC_{50} = 5 µg/ml), *Bifidobacterium* (MIC_{50} = 0.06 µg/ml), *Clostridium* (MIC_{50} = 0.1 µg/ml), *Bacteroides* (MIC_{50} = 0.5 µg/ml), *Lactobacillus* (MIC_{50} = 0.25 µg/ml), *Fusobacterium* (MIC_{50} = 0.1 µg/ml), *Eubacterium* (MIC_{50} = 0.1 µg/ml) and *Peptostreptococcus* (MIC_{50} = 0.1 µg/ml), the MIC_{calc} is 0.10 µg/ml.

Mass of colon content: A value of 220 g is based on the colon content measured from humans.

Fraction of oral dose available to microorganisms: It is recommended that the fraction of an oral dose available for colonic microorganisms be based on in vivo measurements for the drug administered orally. Alternatively, if sufficient data are available, the fraction of the dose available for colonic microorganisms can be calculated as 1 minus the fraction (of an oral dose) excreted in urine. Human data are encouraged, but, in their absence, non-ruminant animal data are recommended. In the absence of data to the contrary, it should be assumed that metabolites have antimicrobial activity equal to that of the parent compound. The fraction may be lowered if the applicant provides quantitative in vitro or in vivo data to show that the drug is inactivated during transit through the intestine. Amoxicillin is rapidly absorbed and is excreted in urine primarily in unchanged form. The lowest amoxicillin urinary recovery data from the human studies was 43.4%. Therefore, the fraction of oral dose available would be 1 – 0.434 = 0.566.

Body weight: The body weight of an adult human is assumed to be 60 kg.

The upper bound of the microbiological ADI for amoxicillin is calculated as indicated below:

$$\text{Upper bound of ADI} = \frac{0.10\ \mu g/ml \times 220\ g}{0.566 \times 60\ kg\ bw}$$

$$= 0.65\ \mu g/kg\ bw$$

Therefore, a microbiological ADI of 0–0.7 µg/kg bw (rounded to one significant figure), or 42 µg for a 60 kg adult, was derived from in vitro MIC susceptibility testing.

4. EVALUATION

As hypersensitivity is frequently encountered in the therapeutic use of penicillins, including amoxicillin, in human medicine, this was considered to be the toxicological effect of concern.

The thirty-sixth meeting of the Committee based its toxicological guidance value of 30 µg/person for benzylpenicillin on only four case-studies of allergy to oral exposure to residues of penicillins, in the absence of information related specifically to benzylpenicillin.

Available limited evidence indicates that, compared with benzylpenicillin and possibly other penicillins, amoxicillin is less chemically reactive with proteins to form haptens. While the Committee recognizes that adverse reactions are generally underreported, it was unable, through a search of the published literature, to identify any cases of allergy following oral exposure to residues of amoxicillin in food. The Committee therefore concluded that the value of 30 µg/person previously established by the thirty-sixth meeting to protect against allergic reactions from residues of penicillins would be unnecessarily conservative in protecting against residues of amoxicillin.

A microbiological ADI of 0–42 µg/person could be established on the basis of disruption of the colonization barrier of the gastrointestinal tract. The Committee therefore concluded that this ADI is sufficient to ensure that allergic reactions would be very unlikely to occur following oral exposure to residues of amoxicillin in food.

The Committee therefore established an ADI of 0–0.7 µg/kg bw on the basis of microbiological effects.

5. REFERENCES

Abou-Tarboush FM (1994). Teratogenic and toxic effects of Hiconcil (amoxicillin) on mouse fetuses. *Arab Gulf Journal of Scientific Research*, 12:133–140.

Acred P et al. (1970). α-Amino-*p*-hydroxybenzylpenicillin (BRL 2333), a new broad-spectrum semisynthetic penicillin: in vivo evaluation. *Antimicrobial Agents and Chemotherapy*, 10:416–422.

Almeida L, Schmauch A, Bergstrom S (1996). A randomised study on the impact of peroral amoxicillin in women with prelabour rupture of membranes preterm. *Gynecologic and Obstetric Investigation*, 41:82–84.

Andrade RJ, Tulkens PM (2011). Hepatic safety of antibiotics used in primary care. *Journal of Antimicrobial Chemotherapy*, 66:1431–1446.

Anfosso F, Leyris R, Charpin J (1979). Drug allergy: in vitro cross-allergenicity between amoxicillin and benzylpenicillin. *Biomedicine*, 30:168–172.

APVMA PUBCRIS (2011). Public Chemical Registration Information System. Australian Pesticides and Veterinary Medicines Authority (http://services.apvma.gov.au/PubcrisWebClient/welcome.do, accessed 7 September 2011).

Arabski M et al. (2005). Interaction of amoxicillin with DNA in human lymphocytes and *H. pylori*–infected and non-infected gastric mucosa cells. *Chemico-Biological Interactions*, 152:13–24.

Arancibia A et al. (1980). Absorption and disposition kinetics of amoxicillin in normal human subjects. *Antimicrobial Agents and Chemotherapy*, 17:199–202.

Beecham Research Laboratories (1969a). BRL 2333: Acute toxicity determinations in mice and rats. Unpublished report from Beecham Research Laboratories, Surrey, England. Submitted to WHO by the United States Food and Drug Administration, Center for Veterinary Medicine.

Beecham Research Laboratories (1969b). Comparative levels of penicillins and penicilloic acid in human urine after oral administration of ampicillin and BRL 2333. Unpublished report from Beecham Research Laboratories, Surrey, England. Submitted to WHO by the United States Food and Drug Administration, Center for Veterinary Medicine.

Beecham Research Laboratories (1970a). BRL 2333: Sub-chronic toxicity study—rats. Unpublished report from Beecham Research Laboratories, Surrey, England. Submitted to WHO by the United States Food and Drug Administration, Center for Veterinary Medicine.

Beecham Research Laboratories (1970b). Urinary excretion after oral and intramuscular administration of BRL 2333 to rats and dogs. Unpublished report from Beecham Research Laboratories, Surrey, England. Submitted to WHO by the United States Food and Drug Administration, Center for Veterinary Medicine.

Beecham Research Laboratories (1971a). Blood level studies of BRL 2333 in dogs. Unpublished report from Beecham Research Laboratories, Surrey, England. Submitted to WHO by the United States Food and Drug Administration, Center for Veterinary Medicine.

Beecham Research Laboratories (1971b). Studies on the in vitro metabolism of BRL 2333 by rat gut contents. Unpublished study no. DM2333/7, Beecham Research Laboratories, Surrey, England. Submitted to WHO by the United States Food and Drug Administration, Center for Veterinary Medicine.

Beecham Research Laboratories (1976). Comparative blood levels between ampicillin and amoxicillin when given orally to chicken, dogs, pigs and calves. Unpublished report from Beecham Research Laboratories, Surrey, England. Submitted to WHO by the United States Food and Drug Administration, Center for Veterinary Medicine.

Benyamini L et al. (2005). The safety of amoxicillin/clavulanic acid and cefuroxime during lactation. *Therapeutic Drug Monitoring*, 27:499–502.

Berg P, Hahn EG (2001). Hepatotoxic reactions induced by β-lactamase inhibitors. *European Journal of Medical Research*, 6:535–542.

Blanca M et al. (1996). Anaphylaxis to penicillins after non-therapeutic exposure: an immunological investigation. *Clinical & Experimental Allergy*, 26:335–340.

Blanca M et al. (1999). Natural evolution of skin test sensitivity in patients allergic to beta-lactam antibiotics. *Journal of Allergy and Clinical Immunology*, 103:918–924.

Blanca M et al. (2001). Clinical evaluation of Pharmacia CAP system RAST FEIA amoxicilloyl and benzylpenicilloyl in patients with penicillin allergy. *Allergy*, 56:862–870.

Bolzan H et al. (2000). [Intrahepatic cholestasis induced by amoxicillin alone.] *Gastroenterología y Hepatología*, 23:237–239 (in Spanish).

Brismar B, Edlund C, Nord CE (1993). Impact of cefpodoxime proxetil and amoxicillin on the normal oral and intestinal microflora. *European Journal of Clinical Microbiology & Infectious Diseases*, 12:714–719.

Cavenee MR et al. (1993). Treatment of gonorrhea in pregnancy. *Obstetrics & Gynecology*, 81:33–38.

Chang CY, Schiano TD (2007). Review article: drug hepatotoxicity. *Alimentary Pharmacology & Therapeutics*, 25:1135–1151.

Chesa-Jiménez J et al. (1994). Low bioavailability of amoxicillin in rats as a consequence of presystemic degradation in the intestine. *Antimicrobial Agents and Chemotherapy*, 38:842–847.

Coker TR et al. (2010). Diagnosis, microbial epidemiology, and antibiotic treatment of acute otitis media in children: a systematic review. *JAMA [Journal of the American Medical Association]*, 304:2161–2169.

Colley DP, Kay J, Gibson GT (1983). Amoxycillin and ampicillin: a study of their use in pregnancy. *Australian Journal of Pharmacy*, 64:107–111.

Comber KR, Osborne CD, Sutherland R (1975). Comparative effects of amoxycillin and ampicillin in the treatment of experimental mouse infections. *Antimicrobial Agents and Chemotherapy*, 7:179–185.

Czeizel AE et al. (2001). Augmentin treatment during pregnancy and the prevalence of congenital abnormalities: a population-based case–control teratologic study. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, 97:188–192.

Delgado S, Flórez AB, Mayo B (2005). Antibiotic susceptibility of *Lactobacillus* and *Bifidobacterium* species from the human gastrointestinal tract. *Current Microbiology*, 50:202–207.

DPD (2011). Drug Product Database online query. Health Canada (http://webprod3.hc-sc.gc.ca/dpd-bdpp/start-debuter.do?lang=eng, accessed 6 September 2011).

Eshelman FN, Spyker DA (1978). Pharmacokinetics of amoxicillin and ampicillin: crossover study of the effect of food. *Antimicrobial Agents and Chemotherapy*, 14:539–543.

Fairclough AF, Filer CW, Smith D (1971). Excretion of radioactivity of penicillin and penicilloic acid following oral administration of ^{14}C-BRL 2333 to rats. Unpublished study no. DM2333/2, Beecham Research Laboratories, Surrey, England. Submitted by the United States Food and Drug Administration, Center for Veterinary Medicine.

FAO/WHO (2011). *Report of the Nineteenth Session of the Codex Committee on Residues of Veterinary Drugs in Foods, Burlington, United States of America, 30 August – 3 September 2010*. Rome, Italy, Food and Agriculture Organization of the United Nations and World Health Organization, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (REP11/RVDF; ftp://ftp.fao.org/codex/Reports_2011/REP11_RVe.pdf).

Filer CW (1971). Biliary and urinary excretion of radioactivity following an oral dose of ^{14}C-BRL 2333 to rats. Unpublished study no. DM2333/5, Beecham Research Laboratories, Surrey, England. Submitted to WHO by the United States Food and Drug Administration, Center for Veterinary Medicine.

Filer CW, Smith D (1971). Excretion of radioactivity following oral administration of ^{14}C-BRL 2333 to dogs. Unpublished study no. DM2333/4, Beecham Research Laboratories, Surrey, England. Submitted to WHO by the United States Food and Drug Administration, Center for Veterinary Medicine.

Garcia Rodriguez LA, Stricker BH, Zimmerman HJ (1997). Risk of acute liver injury associated with the combination of amoxicillin and clavulanic acid. *Archives of Internal Medicine*, 156:1327–1332.

Gordon RC, Regamey C, Kirby WM (1972). Comparative clinical pharmacology of amoxicillin and ampicillin administered orally. *Antimicrobial Agents and Chemotherapy*, 1:504–507.

Haginaka J, Wakai J (1987). Liquid chromatographic determination of amoxicillin and its metabolites in human urine by postcolumn degradation with sodium hypochlorite. *Journal of Chromatography*, 413:219–226.

Hardy TL, Palmer GH, Cozens AC (1971). Acute oral toxicity of BRL 2333 to 3, 22 and 36 day old rats. Unpublished study no. 71/D55, Beecham Research Laboratories, Surrey, England. Submitted to WHO by the United States Food and Drug Administration, Center for Veterinary Medicine.

Hargreaves CA et al. (1998). Effects of co-trimoxazole, erythromycin, amoxycillin, tetracycline and chloroquine on sperm function in vitro. *Human Reproduction*, 13:1878–1886.

Hirakawa T et al. (1983). Reproduction studies in rats. *Chemotherapy*, 31:263–272.

Hussaini SH et al. (2007). Antibiotic therapy: a major cause of drug-induced jaundice in southwest England. *European Journal of Gastroenterology and Hepatology*, 19:15–20.

Istifli ES, Topaktas M (2010). Cytogenetic genotoxicity of amoxicillin. *Environmental and Molecular Mutagenesis*, 51:222–228.

James PA, Hardy TL, Koshima Y (1983). Reproduction studies of BRL 25000. Teratology studies in the pig. *Chemotherapy*, 31:274–279.

Jepsen P et al. (2003). A population-based study of maternal use of amoxicillin and pregnancy outcome in Denmark. *British Journal of Clinical Pharmacology*, 55:216–221.

Kenyon SL, Taylor DJ, Tarnow-Mordi WO (2002). ORACLE—antibiotics for preterm prelabour rupture of the membranes: short-term and long-term outcomes. *Acta Paediatrica (Oslo, Norway : 1992)*, Supplement 91:12–15.

Kenyon SL et al. (2001). Broad-spectrum antibiotics for preterm, prelabour rupture of fetal membranes: the ORACLE I randomized trial. *Lancet*, 357:979–988.

Kounis NG, Giannopoulos S, Goudevenos J (2011). Beware of, not only the dogs, but the passionate kissing and the Kounis syndrome. *Journal of Cardiovascular Medicine*, 12:149–150.

Küng K, Wanner M (1994). Bioavailability of different forms of amoxycillin administered orally to dogs. *The Veterinary Record*, 135:552–554.

Larrey D et al. (1992). Hepatitis associated with amoxycillin–clavulanic acid combination report of 15 cases. *Gut*, 33:368–371.

Li PY et al. (2007). Antibiotic amoxicillin induces DNA lesions in mammalian cells possibly via the reactive oxygen species. *Mutation Research*, 629:133–139.

Lin E, Saxon A, Riedl M (2010). Penicillin allergy: value of adding amoxicillin as a determinant in skin testing. *International Archives of Allergy and Immunology*, 152:313–318.

Lode H et al. (2001). Ecological effects of linezolid versus amoxicillin/clavulanic acid on the normal intestinal microflora. *Scandinavian Journal of Infectious Diseases*, 33:899–903.

Lovett SM et al. (1997). A prospective, double-blind, randomized, controlled clinical trial of ampicillin–sulbactam for preterm premature rupture of membranes in women receiving antenatal corticosteroid therapy. *American Journal of Obstetrics & Gynecology*, 176:1030–1038.

Lucena MI et al. (2006). Determinants of the clinical expression of amoxicillin–clavulanate hepatotoxicity: a prospective series from Spain. *Hepatology*, 44:850–856.

Mancuso G, Berdondini RM (2006). Kiss-induced allergy to amoxicillin. *Contact Dermatitis*, 54:226.

Marmor A, Newman TB (2011). Amoxicillin–clavulanate improves symptoms, reduces treatment failure in select children with acute otitis media and increases risk of diarrhoea. *Evidence-Based Medicine*, 16:150–152.

Mastrandrea V et al. (1984). Human intravenous and intramuscular pharmacokinetics of amoxicillin. *International Journal of Clinical Pharmacology Research*, 4:209–212.

Matanovic SM et al. (2010). Impact of restricted amoxicillin/clavulanic acid use on *Escherichia coli* resistance-antibiotic DU90% profiles with bacterial resistance rates: a visual presentation. *International Journal of Antimicrobial Agents*, 36:369–373.

Mercer BM et al. (1997). Antibiotic therapy for reduction of infant morbidity after preterm premature rupture of the membranes. A randomized controlled trial. National Institute of Child Health and Human Development Maternal–Fetal Medicine Units Network. *JAMA [Journal of the American Medical Association]*, 278:989–995.

Moubareck C et al. (2005). Antimicrobial susceptibility of bifidobacteria. *Journal of Antimicrobial Chemotherapy*, 55:38–44.

Nägele E, Moritz R (2005). Structure elucidation of degradation products of the antibiotic amoxicillin with ion trap MS(n) and accurate mass determination by ESI TOF. *Journal of the American Society of Mass Spectrometry*, 16:1670–1676.

Nahum GG, Uhl K, Kennedy DL (2006). Antibiotic use in pregnancy and lactation. *Obstetrics & Gynecology*, 107:1120–1138.

Nathanson S et al. (2000). In utero and in vitro exposure to β-lactams impair kidney development in the rat. *Journal of the American Society of Nephrology*, 11:874–884.

Noel PR et al. (1967). BRL 2333 & BRL 1957 initial toxicity studies in dogs. Unpublished study no. 2088/67/288, Beecham Research Laboratories, Surrey, England. Submitted to WHO by the United States Food and Drug Administration, Center for Veterinary Medicine.

Noel PR et al. (1970a). BRL 2333 oral toxicity study in Beagle dogs (repeated dosage for six months with interim sacrifice after three months). Unpublished study no. 3338/70/150, Beecham Research Laboratories, Surrey, England. Submitted to WHO by the United States Food and Drug Administration, Center for Veterinary Medicine.

Noel PR et al. (1970b). BRL 2333 acute toxicity study in the Beagle dog. Unpublished study no. 3389/70/201, Beecham Research Laboratories, Surrey, England. Submitted to WHO by the United States Food and Drug Administration, Center for Veterinary Medicine.

O'Donohue J et al. (2000). Co-amoxiclav jaundice: clinical and histological features and HLA class II association. *Gut*, 47:717–720.

Palmer AK, Cozens DD (1969a). Effect of BRL 2333 on pregnancy of the rat. Unpublished study no. 2934/69/360, Beecham Research Laboratories, Surrey, England. Submitted to WHO by the United States Food and Drug Administration, Center for Veterinary Medicine.

Palmer AK, Cozens DD (1969b). Effect of BRL 2333 on pregnancy of the mouse. Unpublished study no. 3125/69/551, Beecham Research Laboratories, Surrey, England. Submitted to WHO by the United States Food and Drug Administration, Center for Veterinary Medicine.

Palmer AK, Cozens DD (1969c). Effect of BRL 2333 on peri- and post-natal development of the rat. Unpublished study no. 3146/69/572, Beecham Research Laboratories, Surrey, England. Submitted to WHO by the United States Food and Drug Administration, Center for Veterinary Medicine.

Palmer AK, Cozens DD (1970). Effect of BRL 2333 on fertility and general reproduction performance of the rat. Unpublished study no. 3301/70/113, Beecham Research Laboratories, Surrey, England. Submitted to WHO by the United States Food and Drug Administration, Center for Veterinary Medicine.

Pétavy-Catala C, Machet L, Vaillant L (2001). Consort contact urticaria due to amoxicillin. *Contact Dermatitis*, 44:251.

PID (2011). Product Information Database. Veterinary Medicines Directorate, Department for Environment, Food and Rural Affairs, United Kingdom (http://www.vmd.defra.gov.uk/ProductInformationDatabase/, accessed 6 September 2011).

Quinlan GJ, Gutteridge JM (1988). Oxidative damage to DNA and deoxyribose by beta-lactam antibiotics in the presence of iron and copper salts. *Free Radical Research Communications*, 5:149–158.

Reyns T et al. (2008). Tissue depletion of amoxicillin and its major metabolites in pigs: influence of the administration route and the simultaneous dosage of clavulanic acid. *Journal of Agricultural and Food Chemistry*, 56:448–454.

Reyns T et al. (2009). Influence of administration route on the biotransformation of amoxicillin in the pig. *Journal of Veterinary Pharmacology and Therapeutics*, 32:241–248.

Ridglan Farms (1975). Subtoxicity study amoxicillin tablets (film-coated)—feline—2/17/75-3/24/75. Unpublished report from Ridglan Farms, Inc., Mt Horeb, WI, USA. Submitted to WHO by the United States Food and Drug Administration, Center for Veterinary Medicine.

Robles M et al. (2010). Antibiotic-induced liver toxicity: mechanisms, clinical features and causality assessment. *Current Drug Safety*, 5:212–222.

Schwarze C et al. (2002). Vanishing bile duct syndrome associated with elevated pancreatic enzymes after short-term administration of amoxicillin. *European Journal of Gastroenterology and Hepatology*, 14:1275–1277.

Sher L et al. (2005). Randomized, investigator-blinded, multicenter, comparative study of gatifloxacin versus amoxicillin/clavulanate in recurrent otitis media and acute otitis media treatment failure in children. *The Pediatric Infectious Disease Journal*, 24:301–308.

Sjövall J, Alván G, Westerlund D (1985). Dose-dependent absorption of amoxycillin and bacampicillin. *Clinical Pharmacology & Therapeutics*, 38:241–250.

Sjövall J, Westerlund D, Alván G (1985). Renal excretion of intravenously infused amoxicillin and ampicillin. *British Journal of Clinical Pharmacology*, 19:191–201.

Spyker DA et al. (1977). Pharmacokinetics of amoxicillin: dose dependence after intravenous, oral and intramuscular administration. *Antimicrobial Agents and Chemotherapy*, 11:132–141.

Stricker BH et al. (1989). Cholestatic hepatitis due to antibacterial combination of amoxicillin and clavulanic acid (augmentin). *Digestive Diseases and Sciences*, 34:1576–1580.

Sutter VL, Finegold SM (1976). Susceptibility of anaerobic bacteria to 23 antimicrobial agents. *Antimicrobial Agents and Chemotherapy*, 10:736–752.

Takata GS et al. (2001). Evidence assessment of management of acute otitis media: 1. The role of antibiotics in treatment of uncomplicated acute otitis media. *Pediatrics*, 108:239–247.

Thiim M, Friedman LS (2003). Hepatotoxicity of antibiotics and antifungals. *Clinics in Liver Disease*, 7:381–399.

Torres MJ et al. (2003). Diagnosis of immediate allergic reactions to beta-lactam antibiotics. *Allergy*, 58:961–972.

Tosoni C et al. (2011). Beware of dog: a case report on cardiac involvement in drug allergy. *Journal of Cardiovascular Medicin*e, 12:686–688.

USCFR (2010). *United States Code of Federal Regulations Title 21, Food and Drug Administration, Sections 520 and 522*. Office of the Federal Register, National Archive and Records Administration. Published by United States Government Printing Office, Washington, DC, USA.

USFDA (2006). *Prescribing information for AUGMENTIN ES-600 (amoxicillin/clavulanate potassium) powder for oral suspension*. United States Food and Drug Administration (http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/050755s016s018lbl.pdf, accessed 20 October 2011).

VICH (2004). *Studies to evaluate the safety of residues of veterinary drugs in human food: general approach to establish a microbiological ADI*. Brussels, Belgium, International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH Guideline 36; http://www.vichsec.org/pdf/05_2004/Gl36_st7_F_rev.pdf).

Vree TB, Dammers E, Exler PS (2003). Identical pattern of highly variable absorption of clavulanic acid from four different oral formulations of co-amoxiclav in healthy subjects. *Journal of Antimicrobial Chemotherapy*, 51:373–378.

Wheldon GH et al. (1968). The toxicity of BRL 2333 administered orally to rats for three weeks. Unpublished study no. 2087/67/287, Beecham Research Laboratories, Surrey, England. Submitted to WHO by the United States Food and Drug Administration, Center for Veterinary Medicine.

Wheldon GH et al. (1970). Toxicity of BRL 2333 to rats during prolonged oral administration over 26 weeks. Unpublished study no. 3413/70/225, Beecham Research Laboratories, Surrey, England. Submitted to WHO by the United States Food and Drug Administration, Center for Veterinary Medicine.

Woodnutt G, Berry V (1999). Two pharmacodynamic models for assessing the efficacy of amoxicillin–clavulanate against experimental respiratory tract infections caused by strains of *Streptococcus pneumoniae*. *Antimicrobial Agents and Chemotherapy*, 43:29–34.

Wren WB (1977). Subacute toxicity study. Amoxi-Ject (amoxicillin trihydrate)—feline. Unpublished report from Beecham Laboratories, Veterinary Medical Division, Bristol, TN, USA. Submitted to WHO by the United States Food and Drug Administration, Center for Veterinary Medicine.