PROCAINE BENZYLPENICILLIN

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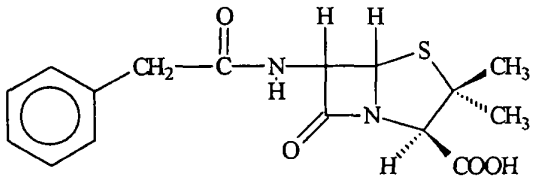
IDENTITY

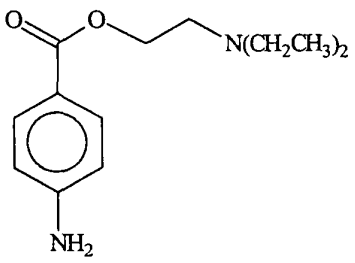
Chemical name: [2S-(2α,5 α,6 β)]-3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-aza-bicyclo-[3.2.0]heptane-2-carboxylic acid compounded with 2-(diethylamino)ethyl 4-aminobenzoate (1:1) monohydrate

Synonyms: benzylpenicillin, benzylpenicillin procaine, procaine penicillin G, Abbocillin-DG, Afsillin, Ampin-penicillin, Aquacillin, Aquasuspen; Avloprocil, Cilicaine, Crysticillin, Despacilina, Despocillin, Distaquaine, Dorsallin "A.R.", Duracillin, Flo-Cillin Aqueous, Hydracillin, Ilcocillin P, Kabipenin, Ledercillin, Lenticillin, Mammacillin, Megapen, Mylipen, Neoproc, Penaquacaine G, Pen-Fifity, Premocillin, Procanodia, Pro-Pen, Wycillin.

Structural formula:



Penicillin G



Procaine

Molecular formula: $C_{29}H_{38}N_4O_6S\text{-}H_2O$

Molecular weight: 588.73

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: procaine benzylpenicillin (penicillin potency approx. 1000 units/mg)

Appearance: monoclinic hemimorphic crystals from methanol-water

Melting point: 106-110°C (with decomposition)

Solubility: Soluble in water, methanol and isopropanol; moderately soluble in ethyl acetate and toluene; slightly soluble in benzene, petroleum ether and carbon tetrachloride; insoluble in iso-octane.

Optical rotation: dextrorotary in aqueous solutions.

Ultraviolet maxima: 190 nm at pH 3.4

Stability: procaine benzylpenicillin is rapidly inactivated by acids, alkalis and oxidizing agents.

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Procaine benzylpenicillin, a combination of two compounds, benzylpenicillin (penicillin G) and procaine (1:1), is one of a number of benzylpenicillin compounds that is widely used in many countries for the treatment and prevention of bacterial infections in cattle, horses, swine, sheep, chickens and turkeys, as well as in various minor species such as rabbits, quail and pheasants. It is available in both injectable and feed additive formulations, frequently in combination with other antimicrobial compounds. Benzylpenicillin is active against gram-positive bacteria, while procaine, one of the first useful local anaesthetics, has been used as such for almost a century.

Benzylpenicillin procaine is hydrolyzed in the muscle to benzylpenicillin and procaine, with subsequent absorption of benzylpenicillin from the muscle. The absorption of procaine is very slow. Usage of procaine benzylpenicillin is based on the prolongation of administration intervals due to the slow absorption of the drug from the injection site.

As procaine benzylpenicillin is poorly soluble in water (4 g/L), the usual formulation for administration is an injectable aluminium monostearate oily suspension. The particle size of the procaine benzylpenicillin in the suspension has considerable effect on the absorption rate of the drug from the injection site. Benzylpenicillin was last reviewed at the 36th Meeting of the Committee.

Dosage

A typical recommended dose by intramuscular injection (IM) in cattle, horses, sheep and swine of a 300,000 unit/mL formulation is 6,600 units/kg BW. As a feed additive, a typical dosage for poultry or swine is 55 mg/kg in the diet. Intramammary treatment is typically by administration of 100,000 units per quarter (Sundlof *et al*, 1988). In the studies reported, 1 mg procaine benzylpenicillin is equivalent to 1667 IU (international units).

METABOLISM

Pharmacokinetics

Toxicological Test Species

No studies involving laboratory animals were reviewed.

Metabolism in Food Animals

Cattle

Five calves (80-110 kg BW) received procaine benzylpenicillin as a single IM injection at a dose of 17760 ± 2325 IU/kg BW (Volner *et al*, 1991). A C_{max} of 2.1 ± 0.6 IU/mL was observed, with an elimination half-life ($t_{½}$) of 4.3 h and AUC of 18.25 ± 4.5 IU/mL/h. Co-administration of phenylbutazone at 24 mg/kg BW increased these parameters by about 20%. Decline in concentrations of benzylpenicillin in plasma was uniphasic.

In six cattle which received procaine benzylpenicillin IM at 10,000 IU/kg BW, in combination with dihydro-streptomycin (12.5 mg/kg BW), the absorption half-life for benzylpenicillin in serum was 9.96 ± 3.84 min., while the elimination half-life ($t_{½}$) was 1.87 ± 0.46 h. (Landoni and Errecalde, 1991). In another study (Bengtsson *et al.*, 1991), six calves (102 - 120 kg BW) received procaine benzylpenicillin at 30 mg/kg BW by IM injection in the neck. Benzylpenicillin administered in this experiment had a $t_{½}$ of 2.98 ± 1.20 h in serum and 10.21 ± 3.45 h in tissue cage fluid. Tissue cages are made from silastic rubber tubing and are implanted subcutaneously to provide a model for the distribution of antibacterial drugs in abscesses. C_{max} , the maximum concentration, was 5.37 ± 2.28 mg/L in serum and 1.52 ± 0.31 mg/L in tissue cage fluid, while the time to maximum concentration, T_{max}, was 1.5 ± 0.78 h in serum and 7.7 ± 2.22 h in tissue cage fluid.

Procaine benzylpenicillin was administered by IM injection to six mature cows at 20,000 IU/kg BW (Conlon *et al.*, 1993). The same 6 cows were administered with the drug 7 days later at the same dose by subcutaneous (SC) injection, repeated on 3 successive days. Blood samples were collected at 3, 5, 10, 15, 20, 30, 45, 60 and 90 min, and at 2, 4, 6, 8, 10, 12 and 24 h

following injection. Peak serum concentration appeared at 30 min after IM administration and at 2 h after SC injection, with a more rapid decline in plasma concentration of benzylpenicillin seen in the first 24 h following SC injection, compared to IM injection. Residues in tissues of the animals which were killed 5 days after the final SC injection were: liver, 1.00 ± 0.80 mg/kg; kidney (renal cortex), 0.90 ± 0.58 mg/kg; kidney (renal medula), 0.58 ± 0.17 mg/kg; muscle (diaphragm), 0.13 ± 0.11 mg/kg; muscle (gluteal), 0.10 ± 0.08 mg/kg; fat, 0.06 ± 0.04 mg/kg; muscle adjacent to injection site, 1.15 ± 1.27 mg/kg.

Two groups of 3 feedlot steers each received once daily on 5 successive days an injection of procaine benzylpenicillin IM of 24,000 or 66,000 IU/kg BW, respectively, with final injection being in the gluteal muscle, while a third group of three animals received a single injection of 66,000 IU/kg BW, IM in the neck, and a fourth group (3 animals) received the same dosage SC (Papich *et al*, 1993). The approved dose is 6,600 IU/kg BW in the United States and 7,500 IU/kg BW in Canada, although higher doses in the range of those used in the study are used in practice. Blood samples were collected at fixed time intervals from 0.25 h to 12 days following final injection and analyzed to determine pharmacokinetic parameters. The highest C_{max} , 4.24 ± 1.08 mg/L, was observed for IM injection of the single dose in the neck, which also provided the shortest $t_{½}$, 8.85 h. Maximum concentration appeared in all cases at 5-6 h following administration. Injection (IM) in the neck gave a higher AUC, 73.03 ± 8.57 μg·h/mL, than IM injection in the gluteal muscle (62.04 ± 3.34 μg·h/mL) or SC injection in the neck (63.15 ± 6.25 μg·h/mL) at the 66,000 IU/kg BW dose. The $t_{½}$ was 15.74 and 15.96 for the IM injections in the gluteal muscle and 18.08 for SC injection in the neck, indicating some variability in the effectiveness of different modes of administration and injection locations.

Another study was reported (Papich *et al*, 1994) in which yearling steers were divided into groups of 4 animals. Group A received an IM injection of a 1:1 mixture of benzathine benzylpenicillin and procaine benzylpenicillin at 9000 IU/kg BW, Group B received an IM injection of 24,000 IU/kg BW of the same mixture in the gluteal muscle, while Group C was administered this mixture as a SC injection of 8,800 IU/kg BW in the neck muscle. Group D received an IM injection of benzathine benzylpenicillin alone in the gluteal muscle at 12,000 IU/kg BW. Blood samples were collected at regular intervals starting at 0.25 h following injection and extending to 14 days. For the combined formulations, C_{max} was observed within 1 to 4 h after treatment, and $t_{½}$ was from 40.6 to 57.7 h for all IM injections. For the SC injection, $t_{½}$ was 29.3 ± h, while for benzathine benzylpenicillin administered alone by IM injection, T_{max} was at 17.6 ± 6.1 h. AUC varied with the dose and mode of injection, being 0.17 μg·d/mL for the SC injection and, for the IM injections, 0.35 μg·d/mL (9000 IU/kg), 0.97 μg·d/mL (24,000 IU/kg) and 0.65 μg·d/mL (benzathine benzylpenicillin only). The results demonstrated the faster uptake of procaine benzylpenicillin relative to benzathine benzylpenicillin and also the variability in pharmacokinetic parameters associated with IM and SC injection of the same product.

Nine calves (149-301 kg BW) were treated in a three-way, randomized crossover experiment with washout periods of at least one week with three different commercially available formulations containing procaine benzylpenicillin and dihydrostreptomycin (Groen *et al.*, 1996). Each formulation, which contained 200,000 IU/mL of procaine benzylpenicillin and 150-200 mg/mL dihydrostreptomycin, was administered at a dose of 0.1 mL/kg BW by IM injection. Pharmacokinetic parameters for benzylpenicillin as measured in serum from blood samples collected from 0.5 to 72 h following treatment were similar for the three formulations, with no statistical difference observed in AUC_{0-t} (mean 13.2- 13.4 μg.h/mL) or $AUC_{0-\infty}$ (mean 13.7-14.0 μg.h/mL). There were statistical differences in the parameters which reflect absorption rate, with $t_{½}$ varying from 5.5 ± 2.7 to 8.3 ± 2.9 h and C_{max} varying from 1.09 ± 0.41 to 1.53 ± 0.48 μg/mL. The formulation which was absorbed at the slowest rate caused the most tissue damage, based on measurements of creatine phosphokinase kinetics.

Horses

Five horses received procaine benzylpenicillin at 20,000 IU/kg BW in 5 different sites of injection: (1) SC in the pectoral area; (2) IM in the belly; (3) IM in musculus serratus ventralis cervicus; (4) IM in the biceps muscle, mid-way between the hip and knee; and (5) IM in the gluteus muscle (Firth *et al*, 1986). Highest $AUC_{0-12\,h}$ (26.0 ± 10.2 IU·h/mL) and shortest $t_{½}$ (8.0 ± 2.7 h) were observed for treatment (3). Subsequently, it was also demonstrated that administration of phenylbutazone boosted levels of benzylpenicillin in plasma when administered concurrently with an IM injection of procaine benzylpenicillin (Firth *et al*, 1990).

Procaine benzylpenicillin was administered IM at 12 mg/kg BW to six ponies and concentrations of benzylpenicillin in plasma and tissue chamber fluid were measured at intervals up to 24 h after treatment (Ensink *et al*, 1996). The AUC in μg·h/mL was 8.8 ± 2.0 for plasma and 4.8 ± 1.7 for tissue chamber fluid, with t_{max} of 3.5 ± 0.8 h and 12.3 ± 9.7 h, respectively. In this study, it was noted that, while renal elimination of benzylpenicillin is rapid, treatment IM with procaine benzylpenicillin can result in a maximum residence time of up to 10 h for benzylpenicillin in plasma. Concentrations in the

tissue chamber fluid exceeded those in the plasma only after C_{max} has been reached and concentrations in both compartments were declining.

Rabbits

Four *P. multocida* free and four infected rabbits each received a single IM injection of procaine benzylpenicillin at 60,000 IU/kg BW (Welch *et al*, 1987). Blood was collected from each animal at 0, 1, 3, 5, 8, 16 and 24 h following injection and nasal washings were collected at 0, 4, 9 and 24 h. Higher concentrations of benzylpenicillin were found in the blood samples collected from the infected rabbits than in the non-infected rabbits in the 1, 3, 5 and 8 h samples. In infected rabbits, the maximum concentration in serum was 5.86 ± 1.09 mg/L at 1 h, declining to 1.90 ± 0.88 mg/L at 8 h. In the non-infected rabbits a maximum concentration of 2.34 ± 1.51 mg/L was seen at 3 h, declining to 0.53 ± 0.24 mg/L at 8 h. A similar profile was observed in nasal washings, where concentrations declined from 0.06 mg/L at 4 h to 0.04 mg/L at both 9 and 24 h in the infected rabbits, but increased from 0.02 mg/L at 4 h to 0.06 mg/L at both 9 and 24 h in non-infected rabbits.

Twelve female rabbits were treated with three commercial products containing procaine benzylpenicillin (120 mg/mL) and dihydrostreptomycin (150-200 mg/mL) in a 4-way, randomized crossover experiment in which the rabbits were divided into 4 groups of 3 animals each (Groen *et al*, 1996). One group received, intravenously, a mixture prepared in the laboratory containing procaine benzylpenicillin and dihydrostreptomycin, while the remaining groups were each treated by IM injection, respectively, with one of the three commercial products. For the benzylpenicillin in the three commercial products, $AUC_{0-\infty}$ was 817 ± 145 to 867 ± 124 μg·min/mL, but $t_{½}$ varied from 111 ± 49 to 374 ± 279 min. C_{max} decreased from 4.4 ± 1.2 to 2.1 ± 0.9 μg/mL as elimination halflife increased, demonstrating that different formulations will have differences in elimination profile.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

No studies using radiolabeled procaine benzylpenicillin were found during the period covered by this literature survey (1984 - 1997).

Other Residue Depletion Studies (with Unlabelled Drug)

Cattle

Six groups (3 animals per group) of yearling steers received an IM injection of 24,000 IU/kg BW procaine benzylpenicillin on 5 successive days, after which the groups were slaughtered, respectively, at 1, 2, 3, 4, 8 and 12 days after the last injection (Korsrud *et al*, 1993). The experiment was repeated using a dose of 66,000 IU/kg BW, but adding two groups of 4 animals each, which were killed, respectively, at 10 and 16 days following the final injection of procaine benzylpenicillin. The results were compared with another experiment in which four groups of 3 yearling steers each received 66,000 IU/kg BW procaine benzylpenicillin by SC injection, again repeated over 5 successive days. Finally, six steers were injected SC with 66,000 IU/kg BW procaine benzylpenicillin, with each steer receiving several injections spaced at timed intervals so that, at the time of slaughter, injection sites were obtained which were 10, 15, 20 or 30 days old. Tissues were analyzed for benzylpenicillin residues using a high performance liquid chromatographic (HPLC) method of analysis with a detection limit of 0.005 mg/kg. Residues in edible tissues resulting from the administration of procaine benzylpenicillin IM at 24,000 and 66,000 IU/kg BW are reported in Table 1. Residues were below the detection limit in many of the injection sites collected at slaughter from the animals treated at 24,000 IU/kg BW and ranged from <0.005 mg/kg to 1.20 mg/kg in injections sites from the animals which received 66,000 IU/kg BW.

There was no clear correlation between time from treatment to slaughter and residues found at the injection site for IM administration. However, in the treatment groups which received procaine benzylpenicillin IM at 66,000 IU/kg BW, two cases of drug entrapment in the musculature at the injection site were encountered. One resulted in residues of 1.20 mg/kg 10 days following treatment, while the other injection site area contained 0.44 mg/kg of benzylpenicillin at 16 days following injection. These injection sites would not have been readily detected in a routine post-mortem inspection and were attributed to the use of injection volumes in excess of 30 mL of the formulated product.

Residues resulting from the SC administration of procaine benzylpenicillin, which were higher than those seen for IM administration, fell to below 0.05 mg/kg in both diaphagm and gluteal muscle 3 days after final treatment, but remained

above this concentration in kidney and liver at 4 days post-treatment. SC administration also resulted in visible deposits of the drug at the injection site at slaughter, with the injection area characterized by edema and haemorrhage. These sites were clearly visible at slaughter and there was a trend to lower residues at the injection site with elapsed time from treatment to slaughter. However, at 10 days following treatment, one injection site was found to have residues of 3.60 mg/kg. As in the case of IM administration, with the exception of injection sites, highest residues were found in the liver, followed by kidney and muscle.

Table 1. Residues in tissues resulting from IM administration of procaine benzylpenicillin at 24,000 or 66,000 IU/kg BW, and from SC administration at 66,000 IU/kg BW, on 5 successive days in yearling steers.

Withdrawal Period (d)	Body Weight (kg)	Dose [1]	Benzylpenicillin Residues in Tissues (mg/kg)			
			Kidney	Liver	Diaphragm Muscle	Gluteal Muscle[2]
1	444±8	A	1.10±0.62	2.00±0.28	0.04±0.00	0.03±0.02
	463±2	B	2.80±0.49	2.30±0.41	0.15±0.02	0.08±0.02
	469±23	C	1.60±0.20	4.70±0.43	0.29±0.02	0.10±0.01
2	494±8	A	0.65±0.39	0.35±0.24	0.03±0.02	0.06±0.03
	465±13	B	1.00±0.34	1.60±0.29	0.05±0.01	0.04±0.02
	466±6	C	0.83±0.18	2.50±0.71	0.07±0.02	0.05±0.00
3	582±10	A	0.03±0.02	0.02±0.02	<0.005	<0.005
	498±11	B	0.24±0.11	0.37±0.18	0.005±0.0050	0.009±0.009
	463±9	C	0.50±0.24	0.88±0.34	0.007±0.004	0.014±0.004
4	421±31	A	0.02±0.00	0.02±0.01	<0.005	<0.005
	451±37	B	0.01±0.01	0.05±0.02	<0.005	<0.005
	472±9	C	0.39±0.10	0.48±0.10	0.013±003	<0.005
8	472±36	A	<0.005	<0.005	<0.005	<0.005
	466±17	B	0.01±0.00	0.07±0.04	<0.005	<0.005
10	550±4	B[3]	0.01±0.01	0.03±0.01	NA[4]	<0.005
12	439±42	A	<0.005	<0.005	<0.005	<0.005
	404±16	B	<0.005	<0.005	<0.005	<0.005
16	607±7	B[3]	0.01±0.01	0.01±0.01	NA[4]	<0.005

[1] Dose: A, 24,000 IU/kg BW IM; B, 66,000 IU/kg BW IM; C, 66,000 IU/kg BW SC
[2] Collected from side of animal where drug was not injected.
[3] These groups contained 4 animals; all others contained 3.
[4] NA indicates "not analyzed".

Subsequently, a depletion study was reported in which cattle were administered either a combination of procaine benzylpenicillin and benzathine benzylpenicillin (1:1) IM at 8,600 IU/kg BW or SC at 8,800 IU/kg BW, or benzathine benzylpenicillin alone IM at 12,000 IU/kg BW (Korsrud *et al*, 1994). Treatment groups were as follows: 10 steers each received a single IM dose of 8600 IU/kg BW benzathine benzylpenicillin - procaine benzylpenicillin (1:1), the approved dose in Canada, and were slaughtered in groups of 5 at 14 and 30 days, respectively, following administration. Five other steers each received a single SC injection of 8800 IU/kg BW benzathine benzylpenicillin - procaine benzylpenicillin (1:1), an approved dose in the United States, and were slaughtered at 30 days following treatment. Fifteen steers were administered 24,000 IU/kg BW benzathine benzylpenicillin - procaine benzylpenicillin (1:1) IM, in two injections of equal volume, separated by 6-8 cm, but in the same general injection site at the same time, after which they were killed in groups of 5 at 8, 14 and 50 days following treatment. Finally, seven steers received 12,000 IU/kg BW benzathine benzylpenicillin,

as 3 injections of equal volume in a triangular pattern, 6-8 cm apart, administered at the same time, following which the steers were killed at day 14. Several untreated steers served as controls. Samples were analyzed using the same HPLC method as in the previous study. Using the recommended dose, benzylpenicillin residues were detectable in liver (0.007 ± 0.004 mg/kg) 14 days following treatment IM of steers with the mixed benzylpenicillins and at 30 days following SC administration (0.013 ± 0.005 mg/kg). Residues in injection sites following IM administration were 3.20 ± 0.72 mg/kg at 14 days and 2.10 ± 0.86 mg/kg at 30 days, while residues at SC injection sites were 1.60 ± 1.00 mg/kg at day 30. At the 24,000 IU/kg BW dose of the mixed benzylpenicillins, residues in IM injection sites were 104.00 ± 55.6 mg/kg at day 14 and 1.20 ± 0.38 mg/kg at day 50. Following SC administration of benzathine benzylpenicillin at 12,000 IU/kg BW, injection sites contained 7.8 ± 1.12 mg/kg benzylpenicillin at day 14. In all treatment groups, residues in liver were below 0.05 mg/kg at day 14. The results demonstrated that benzathine benzylpenicillin results in persistent residues at injection sites when used alone or in combination with procaine benzylpenicillin and that the residues observed were substantially higher than those seen when procaine benzylpenicillin was administered alone, even at higher doses.

Swine

Four groups of 6 market hogs (approx. 90 kg BW) were fed a diet containing a combination of sulfamethazine (330 mg/kg diet), chlortetracycline (330 mg/kg diet) and procaine benzylpenicillin (165 mg/kg diet), a dose 3 times that approved in Canada (Korsrud *et al.*, 1996). The groups were slaughtered, respectively, at withdrawal times of 0, 2, 4 and 8 days, with food access being denied for 5 h prior to slaughter. A fifth group of hogs received non-medicated feed and served as controls. Tissue samples were analyzed by an HPLC method with an LOD of 0.005 mg/kg for liver, kidney and muscle. Benzylpenicillin residues were detected in kidney from only one of the hogs in the zero withdrawal group (0.018 mg/kg), with all other samples from the other hogs in the study containing no detectable penicillin residues.

Groups of six market weight pigs (approx. 90 kg BW) each received IM 15,000 IU/kg BW procaine benzylpenicillin per pig on 3 successive days, following which the groups were slaughtered at 1, 2, 3, 4 and 8 days after final treatment (Korsrud *et al.*, 1998). Two other groups containing 6 and 7 pigs, respectively, received the same treatment and were slaughtered at 5 days after final administration. Samples were analyzed using the same HPLC method as in the previous study with pigs. Highest residues were found in kidney samples, followed by skin, muscle and fat. Liver was not tested for residues in this study. No significant residues were found in injection sites collected from animals slaughtered at 8 days following treatment. Residues found in tissue samples are summarized in Table 2.

Table 2. Benzylpenicillin residues in tissues collected at slaughter from pigs which received procaine benzylpenicillin IM at 15,000 IU/kg BW on three successive days.

Days Post-Treatment	Benzylpenicillin Residues in Tissues (mg/kg)					
	Kidney	Muscle	Skin	Fat	Injection Site (right neck)	Injection Site (left neck)
1	1.30±0.41	0.03±0.01	0.08±0.03	0.01±0.00	---[a]	---[a]
2	0.12±0.07	<0.005	0.02±0.01	<0.005	---[a]	---[a]
3	0.24±0.18	<0.005	<0.015	<0.005	30.0±14.6	---[a]
4	0.006±0.004	<0.005	0.02±0.01	<0.005	0.10±0.08	0.01±0.01
5	0.005±0.005	<0.005	<0.015	<0.005	1.10±1.05	<0.005
5 (repeat)	<0.005	<0.005	<0.015	---[b]	<0.005	0.01±0.01
8	<0.005	<0.005	<0.015	---[b]	<0.005	<0.005

[a] No sample collected; [b] Not analyzed.

Chickens

Four experimental treatments were randomly each assigned to two out of eight pens in which 400 day-old broiler chicks had been distributed at random, 50 chicks per pen (Proudfoot *et al*, 1993). The four treatments, which continued for 42 days until slaughter, were a control diet with no benzylpenicillin added, a diet containing 27.5 mg/kg of procaine benzylpenicillin, a diet with procaine benzylpenicillin provided via drinking water at approximately 27.5 mg/kg diet equivalent, and a replicate of the preceding with the procaine benzylpenicillin concentration reduced by one-half. The

concentrations of benzylpenicillin used in these experiments were significantly higher than the recommended dose of 2.2 mg/kg. Kidney, liver and muscle samples from the birds which were provided the diet which included 27.5 mg/kg procaine benzylpenicillin (Group 2) were tested for benzylpenicillin residues using a thin-layer chromatography-bioautography analytical method with a limit of detection (LOD) of 0.01 mg/kg for benzylpenicillin. No detectable residues were found in any of the tissues.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

The 36th meeting of the Committee, in reviewing benzylpenicillin, concluded that there were good, sensitive bioassay methods for measuring residues in milk and in meat at the concentrations of interest. It was also noted that these methods were not specific for benzylpenicillin and required confirmation by liquid chromatographic methods or mass spectral methods, which had not been demonstrated to have the required sensitivity. This methodology review has therefore been confined to methods published since 1990.

Liquid chromatographic, mass spectral and other physicochemical methods for residue analysis do not distinguish between the various formulated products, which include procaine, benzathine and the sodium and potassium salts of the target analyte, benzylpenicillin. Several extensive reviews have been published since 1990 that provide an excellent summary of the available residue methods (Boison, 1992; Boison, 1995). Of the methods discussed in these references, two were reviewed and accorded provisional status by the Codex Committee on Residues of Veterinary Drugs in Foods. These are, respectively, a gas chromatographic (GC) method of analysis (Meetschen and Petz, 1990) and an HPLC assay (Boison *et al.*, 1991). The HPLC method is based on solid phase extraction and derivatization with 1,2,4-triazole-mercuric chloride, which forms a methylmercaptide derivative of benzylpenicillin which possesses a strong uv-absorbance at 325 nm. Both of these methods were considered to have demonstrated sufficient analytical sensitivity to meet the requirements for regulatory authorities to monitor compliance with the MRLs adopted by the 36th meeting of the Committee.

While an exhaustive review of published methods will not be undertaken in this report, several recent methods, not included in the Boison reviews, should be mentioned. These include an HPLC method for benzylpenicillin residues in milk, with an LOQ of 0.004 mg/L and an average recovery of 82% (Hormazábal and Yndestad, 1995), a method based on gel electrophoresis (Cutting *et al*, 1995) and a multi-residue HPLC method for β-lactams in milk (Moats and Harik-Khan, 1995). In addition, advances in mass spectral equipment and techniques have resulted in improved confirmatory methods which meet the sensitivity requirements for confirmation at the MRLs (Straub *et al*, 1994; Blanchflower *et al*, 1994). Various test kits are also now commercially available for the detection of β-lactams which have the required sensitivity for use in a regulatory program (Boison and MacNeil, 1995).

The major barrier to multi-laboratory validation of analytical methods is the limited stability of benzylpenicillin residues in samples of animal tissues, even when stored at –20°C (Boison *et al*, 1992). In this study, significant loss of residue was observed in samples after 10 days of frozen storage in liver, kidney and muscle tissues frozen without pre-homogenization. Subsequently, it was demonstrated that an accelerated rate of loss of residue occurs in liver samples which have been homogenized prior to storage, with more rapid loss observed in spiked samples than for incurred tissue residues (Gee *et al*, 1996). This latter finding is of particular significance as normal practice for an interlaboratory trial is to prepare sets of samples from homogenized pools of tissue.

APPRAISAL

Procaine benzylpenicillin is one of a number of available formulations of benzylpenicillin, which was previously reviewed by the 12th and 36th meetings of the Committee. A maximum daily intake of 30 μg of residues of benzylpenicillin, based on hypersensitivity reactions of allergic individuals, and MRL's of 0.05 mg/kg for liver, kidney and muscle (all species) and 0.004 mg/kg for milk have been recommended. The 36th meeting of the Committee noted the limited availability of chemical assay methods and made the following recommendations:

1. The provision of further information and the results of new studies on the depletion of residues of benzylpenicillin from the edible tissues of food-producing animals.
2. Investigation of the accuracy and precision of the assays used to measure residues of penicillin.
3. The development of more sensitive chemical assays for benzylpenicillin.

Pharmacokinetic data

In the studies which follow 1 mg of procaine benzylpenicillin equates with 1,667 IU of the drug.

No data were available for review on studies with laboratory animals or on studies using radiolabeled procaine benzylpenicillin, but a number of non-GLP studies involving food animals, some conducted by regulatory authorities, had been published since benzylpenicillin was last reviewed by the Committee.

Cattle In five calves (80-110 kg BW), which received procaine benzylpenicillin as a single IM injection at a dose of approximately 18000 IU/kg BW, the plasma C_{max} was 2.1 IU/mL with an elimination half-life $t_{½}$ of 4.3 h and AUC of 18.25 IU/mL/h. Decline in concentrations of benzylpenicillin in plasma was uniphasic. When six calves (102 -120 kg BW) with implanted tissue cages received procaine benzylpenicillin at 30 mg/kg BW (approx. 30,000 IU/kg BW) by IM injection in the neck, $t_{½}$ was 2.98 h in serum and 10.21 h in tissue cage fluid. C_{max} was 5.37 µg/mL in serum at 1.5 h after injection, and 1.52 µg/mL in tissue cage fluid at 7.7 h.

In six cattle which received procaine benzylpenicillin IM at 10,000 IU/kg BW, in combination with dihydrostreptomycin (12.5 mg/kg BW), the elimination half-life in serum was 1.87 h. When procaine benzylpenicillin was administered IM to six mature cows at 20,000 IU/kg BW, then 7 days later using the same dose by SC injection, repeated on 3 successive days, C_{max} in serum was 30 min after IM administration and 2 h after SC injection. Concentrations of benzylpenicillin in plasma decreased more rapidly in the first 24 h following SC injection, when compared to IM injection. Benzylpenicillin residues in tissues of the animals that were sacrificed 5 days after the final SC injection were distributed as follows: liver, 1.00 mg/kg; kidney (renal cortex), 0.90 mg/kg; kidney (renal medula), 0.58 mg/kg; muscle (diaphragm), 0.13 mg/kg; muscle (gluteal), 0.10 mg/kg; fat, 0.06 mg/kg; muscle adjacent to injection site, 1.15 mg/kg.

In steers which received procaine benzylpenicillin IM or SC at doses up to 66,000 IU/kg BW, in some cases on 5 successive days, the highest C_{max} , 4.24 µg/mL was observed for IM injection of a single dose in the neck. This treatment also provided the shortest plasma elimination half-life, $t_{½}$, of 8.85 h. C_{max} in all treatments occurred within 5-6 h following injection.

The pharmacokinetics of procaine benzylpenicillin used in combination with benzathine penicillin (1:1), administered IM or SC to yearling steers, was compared with IM injection of benzathine benzylpenicillin alone. C_{max} was observed within 1 to 4 h after treatment for the combined formulations, and $t_{½}$ was from 40.6 to 57.7 h for all IM injections. For the SC injection of the combined formulation, $t_{½}$ was 29.3 h. AUC varied with the dose and mode of injection, from 0.17 µg·d/mL for the SC injection to 0.35 - 0.97 µg·d/mL for IM injection of the combined formulation and 0.65 µg·d/mL for benzathine benzylpenicillin alone. The results demonstrated the variability in pharmacokinetic parameters associated with the use of different formulations, and with IM or SC injection of the same product.

When nine calves (149-301 kg BW) were treated in a three-way, randomized crossover experiment with three different commercially available formulations containing procaine benzylpenicillin (200,000 IU/mL) and dihydrostreptomycin (150-200 mg/mL) administered at a dose of 0.1 mL/kg BW by IM injection, no statistical difference was observed in AUC_{0-t} (mean 13.2-13.4 µg·h/mL) or $AUC_{0-\infty}$ (mean 13.7-14.0 µg.h/mL) in serum samples collected from 0.5 to 72 h after treatment. There were significant differences in $t_{½}$, which varied from 5.5 to 8.3 h, and in C_{max}, which ranged from 1.09 to 1.53 µg/mL.

Horses In horses which received procaine benzylpenicillin at 20,000 IU/kg BW IM or SC in different muscle groups, highest AUC_{0-12h} (26.0 IU·h/mL) and shortest $t_{½}$ (8.0 h) were observed for injection in the front shoulder. Six ponies were administered procaine benzylpenicillin IM at 12 mg/kg (equivalent to 12,000 IU/kg) BW, resulting in an AUC, in µg·h/mL, of 8.8 for plasma and 4.8 for tissue cage fluid, with t_{max} of 3.5 h and 12.3 h, respectively. Renal elimination of benzylpenicillin was rapid, with a maximum residence time of 10 h for penicillin in plasma, while concentrations in the tissue cage fluid exceeded those in the plasma only after C_{max} had been reached and concentrations in both compartments were decreasing.

Rabbits The difference in pharmacokinetics of procaine benzylpenicillin in healthy and sick animals was demonstrated when *P. multocida* free and infected rabbits received a single IM injection of procaine benzylpenicillin at 60,000 IU/kg BW. Higher concentrations of benzylpenicillin were found in blood samples collected from the infected rabbits than from the non-infected rabbits up to 8 h following treatment. In infected rabbits, serum C_{max} was 5.86 µg/mL at 1 h, declining to 1.90 µg/mL at 8 h, while in the non-infected rabbits C_{max} was 2.34 µg/mL at 3 h, declining to 0.53 µg/mL at 8 h. When rabbits

were treated with thee commercial products containing procaine benzylpenicillin and dihydrostreptomycin in a 4-way, randomized crossover experiment similar to the study with calves, $t_{½}$ varied from 111 to 374 min and C_{max} decreased from 4.4 to 2.1 g/mL as elimination halflife increased, demonstrating again that different formulations provide differences in elimination profile.

No information was available on the pharmacokinetics following oral administration of procaine benzylpenicillin.

Residue data

Cattle A study was conducted in which yearling steers received an IM injection of 24,000 or 66,000 IU/kg BW procaine benzylpenicillin on 5 successive days, or 66,000 IU/kg BW procaine benzylpenicillin by SC injection, again repeated over 5 successive days. In addition, another group of steers received procaine benzylpenicillin SC at 66,000 IU/kg BW, with each steer receiving several injections spaced at timed intervals so that injection sites were collected which were 10, 15, 20 or 30 days old at slaughter. Tissues were analyzed for benzylpenicillin residues using a liquid chromatographic method of analysis with a detection limit of 0.005 mg/kg. Mean residues were <0.05 mg/kg in all tissues for the 24,000 IU/kg BW treatment group at day 4, while 10 days were required to reach this concentration range for the 66,000 IU/kg BW IM treated animals. Residues were more persistent following SC injection at 66,000 IU/kg BW, with observed distribution as follows at 4 days withdrawal: liver, 0.48 mg/kg; kidney, 0.39 mg/kg; diaphragm muscle, 0.013 mg/kg; gluteal muscle, <0.005 mg/kg. Residues were <0.005 mg/kg in many of the injection sites collected at slaughter from the animals treated at 24,000 IU/kg BW and ranged from <0.005 mg/kg to 1.20 mg/kg in injection sites from the animals which received 66,000 IU/kg BW. While there was no clear relationship between time from treatment to slaughter and residues found at the injection site following IM administration, several instances of drug entrapment in the musculature at the injection site were noted, one resulting in residues of 1.2 mg/kg at 10 days following treatment, while another injection site contained 0.44 mg/kg of benzylpenicillin at 16 days following the final injection. These injection sites would not have been readily detected in a routine post-mortem inspection and were attributed to the use of injection volumes in excess of 30 mL of the formulated product. Administration of the drug by SC resulted in visible deposits of the drug at the injection site at slaughter, with the injection area characterized by edema and haemorrhage. Excluding injection sites, highest residues were found in the liver, followed by kidney and muscle, for both IM and SC injection.

In a subsequent study, cattle were administered either a combination of procaine benzylpenicillin and benzathine benzylpenicillin (1:1) IM or SC, or benzathine benzylpenicillin alone IM. Using the label dose (8,600 IU/kg BW IM or 8,800 IU/kg BW SC), residues were detectable in liver (0.007 mg/kg) 14 days following IM treatment of steers with the mixed penicillins and at 30 days following SC administration (0.013 mg/kg). Residues in injection sites following IM administration were 3.20 mg/kg at 14 days and 2.10 mg/kg at 30 days, while residues at SC injection sites were 1.60 mg/kg at day 30. At a dose of 24,000 IU/kg BW IM of the mixed penicillins, residues in injection sites were 104 mg/kg at day 14 and 1.2 mg/kg at day 50. Following SC administration of benzathine penicillin alone at 12,000 IU/kg BW, injection sites contained 7.8 mg/kg penicillin at day 14. In all treatment groups, residues in liver were below 0.05 mg/kg at day 14. The findings demonstrated that the risk of persistent residues at injection sites increases when high doses of long-acting formulations are administered.

Pigs In pigs (approx. 90 kg BW) fed a diet containing a combination of sulfamethazine (330 mg/kg diet), chlortetracycline (330 mg/kg diet) and procaine benzylpenicillin (165 mg/kg diet), benzylpenicillin residues were detected in kidney from only one of the hogs in the zero withdrawal group (0.02 mg/kg), with all other tissue samples containing no detectable benzylpenicillin residues. Analysis was by a liquid chromatographic method with an LOD of 0.005 mg/kg for liver, kidney and muscle.

In another study, pigs (approximately 90 kg BW) each received IM 15,000 IU/kg BW procaine benzylpenicillin per pig on 3 successive days. Groups were sacrificed at 1, 2, 3, 4, 5 and 8 days after final treatment. Residues were determined using a liquid chromatographic method with a LOD of 0.005 mg/kg in tissues. Highest residues were found in kidney samples, ranging from 1.30 mg/kg at day 1 to 0.24 mg/kg at day 3 and <0.005 mg/kg at day 8. The only other tissue samples in which residues exceeded 0.05 mg/kg was skin at day 1 (0.08 mg/kg). Liver was not tested for residues in this study and no residues above the MRL were found in injection sites collected from animals slaughtered at 4-8 days following treatment.

Chickens Day-old broiler chicks received a diet for 42 days containing 27.5 mg/kg of procaine benzylpenicillin, or an equivalent dose via drinking water, with no detectable residues found in any of the tissues (kidney, liver, muscle) tested using a thin-layer chromatography-bioautography analytical method with a limit of detection (LOD) of 0.01 mg/kg. The concentrations of benzylpenicillin used in these experiments were significantly higher than the recommended rate of 2.2 mg/kg.

Analytical methods

The 36th meeting of the Committee noted the availability of bioassay methods for measuring benzylpenicillin residues in milk with detection limits between 0.001 and 0.010 mg/L. Such methods were also available for residues in tissues at the concentrations of interest, but the Committee also observed that these methods were not specific for benzylpenicillin and required confirmation by liquid chromatographic methods or mass spectral methods. The available chemical methods had detection limits of 0.05-0.10 mg/kg for tissues and 0.01-0.05 mg/kg for milk, so lacked the required sensitivity.

The present Committee noted that for liquid chromatography, mass spectrometry and other physicochemical methods for residue analysis, the target analyte is typically benzylpenicillin. These methods do not usually distinguish between the various formulated products, which include procaine, benzathine and the sodium and potassium benzylpenicillin salts. It was further noted that several comprehensive reviews have been published since 1990 which provide an excellent picture of the available residue methods for benzylpenicillin, and that two methods have met the criteria for provisional methods established by the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF)(Vol. 3, Codex Alimentarius). Both of these methods were considered to have demonstrated sufficient analytical sensitivity to meet requirements for regulatory authorities accepting the MRL's adopted by the 36th meeting of the Expert Committee. The Committee considered that a number of methods may now be found in the scientific literature, using various analytical techniques, including liquid chromatography and gel electrophoresis, and that some of these methods have limits of detection of 0.002 mg/L for milk and 0.005 mg/kg or less for edible tissues. Some such methods may be suitable for further consideration by the CCRVDF. It also noted that there have been significant improvements in the sensitivity of mass spectrometry techniques, making confirmation of residues at the recommended MRLs of 0.004 mg/L in milk and 0.05 mg/kg in edible tissues feasible with current equipment. A variety of rapid test techniques using bioassay or ELISA are also available for screening purposes.

The Committee, however, noted that a major barrier to multi-laboratory validation of analytical methods is the limited stability of benzylpenicillin residues in samples of animal tissues, even when these tissues are stored at -20 C. For compounds where stability is an impediment to multi-laboratory validation of a method using an exchange of samples, alternative approaches using data individually generated in multiple laboratories, or validation using other criteria acceptable to the CCRVDF, should be considered.

National regulatory authorities should note that different formulations and modes of administration, as well as the use of extra-label doses, may result in more persistent residues in excess of the MRLs, particularly in organ tissues and at injection sites when slow release formulations are used. Suitable analytical methods are available for application as screening, determinative or confirmatory tests in a regulatory monitoring program.

Maximum Residue Limits

The Committee considered that MRLs established by the 36th meeting of the Committee for benzylpenicillin remain appropriate and are applicable to residues resulting from the use of procaine benzylpenicillin. The MRL for liver, kidney and muscle in cattle, pigs and chickens is 50 μg/kg and 4 μg/L for milk. Based on available data, the recommended tissues for regulatory monitoring are kidney or liver, while muscle is an appropriate target tissue for testing for international trade purposes. Procaine benzylpenicillin is also used in horses, sheep, turkeys, rabbits, quail, and pheasants. Due to the lack of information, MRLs could not be established for those species.

REFERENCES

Bengtsson, B., Franklin, A., Jacobsson, S., Luthman, J., and Horn af Rantzien, M. (1991). Distribution of penicillin-G and spiramycin in tissue cages and subcutaneous tissue fluid in calves. *Research in Veterinary Science,* **50**: 301-307.

Boison, J.O., Salisbury, C.D.C., Chan, W., and MacNeil, J.D. (1991). Determination of penicillin G residues in edible animal tissues by liquid chromatography. *J. Assoc. Offic. Anal. Chem.* **74**: 497-501.

Boison, J.O. (1992). Chromatographic methods of analysis for penicillins in food-animal tissues and their significance in regulatory programs for residue reduction and avoidance. *J. Chomatogr.* **624**: 171-194.

Boison, J.O., Korsrud, G.O., MacNeil, J.D., Yates, W.D.G., and Papich, M.G. (1992). Effect of cold-temperature storage on stability of benzylpenicillin residues in plasma and tissues of food-producing animals. *J. AOAC Int.* **75**: 974-978.

Boison, J.O. (1995). "Chemical Analysis of β-Lactam Antibiotics" in *Chemical Analysis for Antibiotics Used in Agriculture* , Oka, H., Nakazawa, H., Harada, K.-I., and MacNeil, J.D., (Editors), ISBN 0-935584-57-9, AOAC International, Gaithersburg, MD 20877-2504, pp.235-306.

Boison, J.O., and MacNeil, J.D. (1995). "New Test Kit Technology" in *Chemical Analysis for Antibiotics Used in Agriculture* , Oka, H., Nakazawa, H., Harada, K.-I., and MacNeil, J.D., (Editors), ISBN 0-935584-57-9, AOAC International, Gaithersburg, MD 20877-2504, pp.77-119.

Conlon, P.D., Butler, D.G., Burger, J.P., and Gervais, M.D. (1993). Evaluation of route and frequency of administration of three antimicrobial drugs in cattle. *Can. Vet. J.* **34**: 606-610.

Cutting, J.H., Kiessling, W.M., Bond, F.L., McCarron, J.E., Kreuzer, K.S., Hurlburt, J.A., and Sofos, J.N. (1995). Agarose gel electrophoretic detection of six β-lactam antibiotic residues in milk. *J. AOAC Int.* **78**:663-667.

Firth, E.C., Nouws, J.F.M., Driessens, F., Schmaetz, P., Peperkamp, K., Klein, W.R. (1986). Effect of the injection site on the pharmacokinetics of procaine penicillin G in horses. *Am. J. Vet. Res.* **47**: 2380-2384.

Firth, E.C., Nouws, J.F.M., Klein, W.R., and Driessens, F. (1990). The effect of phenylbutazone on the plasma disposition of penicillin G in the horse. *J. Vet. Pharmacol. Therap.* **13**: 179-185.

Gee, H.-E., Ho, K.-B., and Toothill, J. Liquid chromatographic determination of benzylpenicillin and cloxacillin in animal tissues and its application to a study of the stability at –20°C of spiked and incurred residues of benzylpenicillin in ovine liver. *J. AOAC Int.* **79**: 640-644.

Groen, K., Mevius, D.J., Pereboom-De Fauw, D.P.K.H., De Neeling, A.J., and Vulto, A.G. (1996). Bioequivalence study in calves of three commercial penicillin/dihydrostreptomycin fixed combination products for intramuscular injection. *J. Vet. Pharmacol. Therap.*, **19**: 370-375.

Groen, K., Pereboom-De Fauw, D.P.K.H., Van Veen-Rutgers, A., Vulto, A.G., and De Neeling, A.J. (1996). Bioavailability in the rabbit of penicillin and dihydrostreptomycin from three commercial penicillin/aminoglycoside fixed combination products for intramuscular injection. *J. Vet. Pharmacol. Therap.*, **19**: 364-369.

Hormazábal, V., and Yndestad, M. (1995). Detection of benzylpenicillin in milk by HPLC. *J. Liq. Chomatogr.* **18**: 2467-2474.

Korsrud, G.O., Boison, J.O., Papich, M.G., Yates, W.D.G., MacNeil, J.D., Janzen, E.D., Cohen, R.D.H., Landry, D.A., Lambert, G., Yong, M.S., and Messier, J.R. (1993). Depletion of intramuscularly and subcutaneously injected procaine penicillin G from tissues and plasma of yearling beef steers. *Can. J. Vet. Res.* **57**: 223-230.

Korsrud, G.O., Boison, J.O., Papich, M.G., Yates, W.D.G., MacNeil, J.D., Janzen, E.D., MacKinnon, J.J., Landry, D.A., Lambert, G., Yong, M.S., and Ritter, L. (1994). Depletion of penicillin G residues in tissues and injection sites of yearling beef steers dosed with benzathine penicillin G alone or in combination with procaine penicillin G. *Food Additives and Contaminants* **11**: 1-6.

Korsrud, G.O., Papich, M.G., Fesser, A.C.E., Salisbury, C.D.C., and MacNeil, J.D. (1996). Residue depletion in tissues and fluids from swine fed sulfamethazine, chlortetracycline and penicillin G in combination. *Food Additives and Contaminants,* **13**: 287-292.

Korsrud, G.O., Salisbury, C.D.C., Rhodes, C.S., Papich, M.G., Yates, W.D.G., Bulmer, W.S., MacNeil, J.D., Landry, D.A., Lambert, G., Yong, M.S., and Ritter, L. (1998). Depletion of penicillin G residues in tissues, plasma and injection sites of market pigs injected intramuscularly with procaine penicillin G. *Food Additives and Contaminants*, **15**: 421-426.

Landoni, M.F., and Errecalde, J.O. (1991). Pharmacokinetic study of a procaine penicillin-dihydrostreptomycin combination in cattle. *Revista-de-Medicina-Veterinaria-Buenos-Aires*, **72**: 198-200.

Meetschen, U., and Petz, M. (1990). Sensitive confirmatory method for determination of seven penicillins in bovine tissues and milk, *Proceedings of the Euro Residue Conference on Residues of Veterinary Drugs in Food, Noordwijkerhout, May 21-23*, Haagsma, N., Ruiter, A., and Czedik-Eysenberg, P.B. (Editors), Addix, Wijk bij Duurstede, pp. 267-271.

Moats, W. A., and Harik-Khan, R. (1995). Liquid chromatographic determination of β-lactam antibiotics in milk: a multiresidue approach. *J. AOAC Int.* **78**: 49-54.

Papich, M.G., Korsrud, G.O., Boison, J.O., Yates, W.D.G., MacNeil, J.D., Janzen, E.D., Cohen, R.D.H., and Landry, D.A. (1993). A study of the disposition of procaine penicillin in feedlot steers following intramuscular and subcutaneous injection. *J. Vet. Pharmacol. Therap.* **16**: 317-327.

Papich, M.G., Korsrud, G.O., Boison, J.O., Yates, W.D.G., MacNeil, J.D., Janzen, E.D., McKinnon, J.J., and Landry, D.A. (1994), Disposition of penicillin G after administration of benzathine penicillin G or a combination of benzathine penicillin G and procaine penicillin G in cattle. *Am. J. Vet. Res.* **55**: 825-830.

Proudfoot, F.G., Hamilton, R.M.G., Jackson, E.D., Hulan, H.W., and Salisbury, C.D.C. (1993). Effects of route and level of administration of procaine penicillin on the performance of broiler chicks. *Can. J. Anim. Sci.* **73**: 141-147.

Sundlof, S.F., Riviere, J.E., and Craigmill, A.L. (1988). *Food Animal Residue Avoidance Data Bank Trade Name File: A Comprehensive Compendium of Food Animal Drugs.* Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida, pp. 271-290.

Volner, Z., Nouws, J., Primozic, S., and Kozjek, F. (1991). Pharmacokinetics of procaine penicillin G administered alone or in co-administration with phenylbutazone in calves. *Acta veterinaria Scandinavica Supplementum*, **87**: 127-128.

Welch, W.D., Lu, Y.-S., and Bawdon, R.E. (1987). Pharmacokinetics of penicillin-G in serum and nasal washings of *Pasteurella multocida* free and infected rabbits. *Lab. Anim. Sci.* **37**: 65-68.