GENTAMICIN

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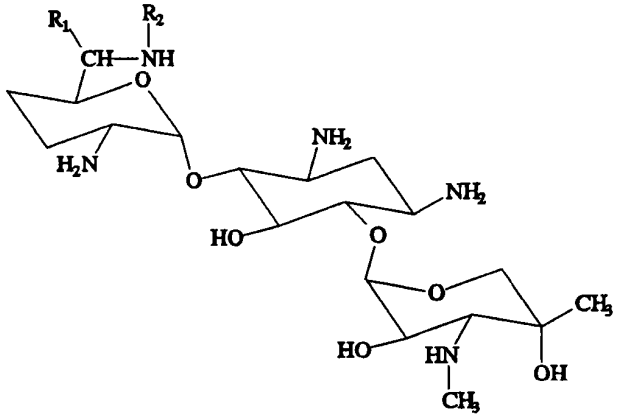
IDENTITY

Chemical name: Gentamicin

Synonyms: Gentamam, Septigen, Bigental, Uterogen, Gentocin, Gentavetina (Product Trade Names)

Structural formula:



Gentamicin C_1 :R_1 = R_2 = CH_3
Gentamicin C_2 :R_1 = CH_3 ; R_2 = H
Gentamicin C_{1a} :R_1 = R_2 = H

Molecular formula: $C_{19\text{-}21}H_{39\text{-}41}N_5O_7$

Molecular weight: 475.9 (C_1, 477.6; C_2, 463.6; C_{1a}, 449.5)

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:

Appearance:	White to buff-coloured powder (as sulphate)
Melting point:	218-237° (as sulphate), 102-108° (pure complex)
Solubility:	Freely soluble in water; soluble in pyridine, dimethylformamide and in acidic media with salt formation; moderately soluble in acetone, ethanol and methanol; virtually insoluble in benzene and halogenated hydrocarbons (pure complex); freely soluble in water, 0.1N hydrochoric acid and 0.1N sodium hydroxide; soluble in ethylene glycol and formamide; practically insoluble in alcohol, choroform and ether (as sulphate).
Optical rotation:	[a]S(25,D) +146° (pure complex) [a]S(25,D) +102° (as sulphate)

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Gentamicin, an aminoglycoside antibiotic produced by fermentation of *Micromonospura purpurea*, is a mixture of basic, water soluble compounds containing the aminocyclitol 2-deoxystreptamine and 2 additional amino sugars. The three major active components are designated C_1, C_2 and C_{1a}, but other minor active components which may be present include gentamicins A, B, B_1 and X. The gentamicin C complex is normally formulated as the sulfate salt, a white-to-buff colored, odourless, water-soluble powder. Ion exchange procedures are frequently used for the separation and purification of gentamicin on a commercial scale, after pH adjustment of the fermentation broth and filtration. Gentamicin is indicated for the treatment of a variety of susceptible bacterial infections in swine, poultry, bovines and equines (eg., colibacillosis and peritonitis in swine; for mastitis, urinary tract disease, respiratory disease and septicemia in bovines). Gentamicin has not been previously reviewed by the Committee.

Dosage

Various formulations of gentamicin have been developed for the treatment of food producing animals, some of these in combination with other antibiotics such as penicillin G, ampicillin and cloxacillin. Formulated products typically contain from 4 - 80 mg/ml of gentamicin and are available for administration by injection, mammary infusion, oral treatment or as an additive to drinking water. Directions for use vary from one time only for the oral treatment to 1-2 injections per day for up to 3 days for injectables to 1-3 treatments for mastitis products. Typical treatment time with the drinking water additive formulation is 3 days.

Gentamicin is currently used in the following food producing animals species : swine, poultry, equine and bovine including lactating cows.

DOSAGE	VIA	TARGET SPECIES
2.5-4 mg/kg one /several treatments	oral and injection	piglets pigs
2-4 mg/kg/day	injection	1-day-old chicks
100 mg/quarter/day	infusion	lactating cows
2-5 mg/kg/day	injection	cattle
2-5 mg/kg/day	injection	equine

METABOLISM

General

Data were considered from studies of the radio-labelled drug in a beagle dog, in 3-day-old piglets and in weanling piglets. Studies with non-labelled gentamicin have also been conducted in a variety of species, including pigs, cattle and chickens. After oral administration, gentamicin is essentially non-absorbed. It is, however, well-absorbed after intramuscular or subcutaneous injection and is excreted unchanged via the kidney. In general, aminoglycosides distribute primarily to the extracellular fluid space and accumulate in the kidney (Riviere et al., 1991).

Dog

A beagle dog which received a single intravenous dose of ^{14}C-labelled gentamicin (9.4 mg/kg BW) excreted approximately two-thirds of the dose in 24-hour urine, while only traces were found in the faeces (Miller et al., 1976). The remainder of the dose was eliminated much more slowly with trace quantities present in urine 31 days post-treatment and 30% of the dose still unaccounted for. Changes in the elimination rate calculated from the urine data suggested depletion via a multi-compartment model. Consistent results were obtained for levels of gentamicin in serum collected from a second beagle which received a dose of 20 mg/kg BW (Miller et al., 1976). Three different measurement methods, total radioactivity, microbiological assay and radioimmunoassay, produced similar results which suggested that no significant metabolism occurred in the dog. Microbiological assays of urine samples provided higher results for most samples than radioactive counts, but the total gentamicin recovery for all samples tested was in good agreement. At sacrifice of the second beagle 8 days after dosing, gentamicin was detectable in all organ tissues tested by total radioactivity, with most of the residues found concentrated in the kidney cortex (approximately 400 times higher than in the skeletal muscle).

Swine

Twelve 3-month old female pigs were divided into two groups, each of which received 200 mg gentamicin oral solution or soluble powder via intubation for 3 consecutive days, in a crossover design. The estimated dose was 6.35 mg/kg BW/d, based on the average initial weights of the animals used in the study. Both formulations produced sub-therapeutic levels in plasma, with no significant difference in the concentrations attained in plasma using either formulation (Lamendola et al., 1980).

Poultry

In a study in which day-old chicks were treated with 0.2 mg gentamicin by subcutaneous injection, gentamicin was rapidly absorbed and reached peak levels in the blood within 30 min (Bickford, 1975). Rapid excretion followed, with blood levels dropping to one-half peak values within two hours. Gentamicin activity was measured in the rectum and duodenum through 24 hours, through 72 hours in the lungs and heart and to 7 days in the bile and yolk sac after this single treatment. Distribution was rapid throughout the tissues, reaching a peak level of 50.4 mg/kg in kidney 24 hours after treatment and declining to 3.3 mg/kg in kidneys at 7 days.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

Three-day-old piglets treated orally with 5 mg ^{3}H-gentamicin (dosage approximately 3.6 mg/kg BW/d) were sacrificed at 1, 3, 6, 11, 14 and 17 days post-treatment, using 3 treated piglets and 1 control per slaughter date (Pavelchak and Rock, 1979). Samples of liver, kidney, muscle and fat were collected and analyzed by total reactivity. The results, shown in Table 1, demonstrated that gentamicin persists for the longest period at the highest levels in kidney and showed little distribution of the drug into muscle tissue. No residues were detectable at 11 days, except in one kidney which contained 0.11 mg/kg of gentamicin.

Table 1. ^{3}H-gentamicin residues in swine tissues following oral administration of 5 mg to 3-day-old piglets[a], expressed as mg/kg

Days Withdrawal	Kidney	Liver	Muscle	Fat
1	4.73	0.20	<0.05	0.05[b]
3	0.44	0.06[c]	<0.05	<0.05
6	0.31	0.10[d]	<0.05	<0.05
11	*[e]	<0.05	<0.05	<0.05
14	<0.05	<0.05	<0.05	<0.05
17	<0.05	<0.05	<0.05	<0.05

[a] Average for three animals, 5 replicate assays per tissue per animal, assay limit of detection 0.05 mg/kg.
[b] Based on samples from only one animal.
[c] Average excludes samples from two animals that contained <0.05 mg/kg gentamicin.
[d] Average includes one sample containing 0.11 mg/kg gentamicin.
[e] Individual values <0.05, 0.11, <0.05 mg/kg gentamicin.

Gentamicin levels in kidneys from 6-week-old pigs treated intramuscularly (Rock et al., 1978 a) or orally (Rock et al., 1978 b) with ^{3}H-labelled gentamicin were analyzed by total radioactivity, microbiological assay and radioimmunoassay, with all assays producing similar results, thereby suggesting that the gentamicin was not significantly metabolized. In the piglets administered gentamicin, 50 mg/gallon drinking water *ad libitum*, formulated to include ^{3}H-labelled drug, 4.27 μCi/mmole, were calculated to have consumed an average 5.37 $\pm$ 1.20 mg/lb (0.81 mg/kg BW/d) of gentamicin during the 3-day treatment period (Rock et al., 1978 a). Three piglets were slaughtered at each of days 1, 3, 5 and 7 post-treatment. Results of the total radioactivity assay are presented in Table 2.

Table 2. ^{3}H-gentamicin residues in swine tissues following administration in water (50 mg/gal) *ad libitum* for 3 days to 6-week-old piglets[a], expressed in mg/kg

Withdrawal (days)	Kidney	Liver	Muscle	Fat
1	0.18	0.11	<0.03	<0.03
3	0.06	0.08	<0.03	<0.03
5	<0.04[b]	0.05	<0.03	<0.03
7	<0.04[c]	0.04	<0.03	<0.03

[a] Average for three animals, 5 replicate assays per tissue per animal.
[b] Average includes results for one kidney that contained <0.03 mg/kg gentamicin.
[c] Average includes results for two kidneys that each contained 0.04 mg/kg gentamicin.

3-Day old piglets which received a single 5 mg intramuscular injection of ^{3}H-gentamicin administered in the mid-region of the right semitendinosus muscle were sacrificed at 14, 28, 35, 42 or 49 days after medication (Rock et al., 1978 b). Samples of kidney, liver, fat, muscle and injection site were analyzed by the three methods described in the previous experiment. Again, the close agreement of the three assay methods used suggested no significant metabolism, as the presence of metabolites should lead to differences in the analytical results obtained with the different assay methods. Highest concentrations of gentamicin were found in kidney for all collection dates and animals tested, as shown in Table 3.

Table 3. ^{3}H-gentamicin residues (mg/kg) in swine tissues following administration of a single intramuscular injection (5 mg) to 3-day-old piglets[a]

Withdrawal (d)	n	Kidney	Liver	Inj. Site	Muscle	Fat
14	2	0.68	0.42	0.12	<0.02	<0.02
28	3	0.18	0.11	<0.02	<0.02	<0.02
35	3	0.07	0.06	<0.02	<0.02	<0.02
42	3	0.05	0.04	<0.02	<0.02	<0.02
49	1	0.02	0.02	<0.02	<0.02	<0.02

[a] Based on five replicate assays per tissue sample

Other Residue Depletion Studies (with Unlabeled Drug)

Cattle

Gentamicin was administered to recently weaned calves at 4 mg/kg BW/day by IM injection in the neck on three successive days (Banting, 1982 a). The calves were divided into groups of 3-5 which were slaughtered, respectively, at 7, 30, 60, 70 and 80 days after the final treatment. Muscle, liver and kidney samples were collected for all dates, and injection site samples were collected from the 30-day and 60-day groups. The samples were analyzed using a bioassay with a limit of detection of 0.05 mg/kg for gentamicin. Residues were present in all kidney samples tested, ranging from >10 mg/kg at day 7 to 0.45-0.75 mg/kg at day 80. Detectable residues were present in 2 of 3 liver samples at day 70 (0.35, 0.50 mg/kg) and in one of 3 liver samples from day 80 (0.10 mg/kg). One muscle sample from day 7 contained 3.6 mg/kg of gentamicin, while all other muscle samples, including those from injection sites, contained no detectable residues. This experiment was confounded by illness of the calves and the death of seven of the experimental animals. Results of this study are summarized in Table 4.

Table 4. Gentamicin residues in calf tissues as determined by cylinder plate assay following intramuscular administration of a single dose of 4 mg/kg BW, expressed as mg/kg[a].

Withdrawal (d)	n	Kidney	Liver	Inj. Site	Muscle
7	5	>10	3.6	na[b]	nd[c]
30	3	2.0	0.8	nd	nd
60	5	1.1	0.6	nd	nd
70	3	0.9	0.3	na	nd
80	3	0.6	0.03	na	nd

[a] Assay limit of detection 0.05 mg/kg
[b] na = not analyzed
[c] nd = not detected

Twenty weaned calves were treated with 4 mg/kg BW by both intramuscular and oral dose, followed 12 hours later by an additional oral dose of 4 mg/kg BW (Pavelchak and Lamendola, 1980 a). Three calves and one control were slaughtered at each of 70, 80, 90 and 100 days of withdrawal and samples of kidney, liver, muscle and fat were collected. Only kidney tissue was analyzed. At 70 days, average residues in kidneys, determined by radioimmunoassay, were 0.48 ± 0.25 mg/kg and averaged less than 0.3 mg/kg at other sampling dates. Kidney samples from one animal killed at 100 days, which was consistently ill and had poor weight gain during the experiment, contained 0.47 mg/kg gentamicin. This result may be more representative of the persistence of residues in sick animals than the residues observed in the healthy animals in the study.

Five mature dairy cows were also treated with gentamicin, at 4 mg/kg BW/day, for three successive days, by IM injection in the neck, using several injection sites per treatment (Banting et al, 1982 b). Milk samples were collected and analyzed as above at 24, 38, 46, 60, 68, 82 and 90 hours following the last treatment. One sample of milk taken at 46 hours contained 0.07 mg/kg gentamicin, while all others were negative showing results below the detection limit, 0.05 mg/kg.

Three studies report residues resulting from the administration of gentamicin by intramammary infusion (Spreat and Riggs, 1978; Carver et al., 1981; Spreat and Lobell, 1980). Five normal mature lactating cows received 100 mg of gentamicin in each quarter immediately after each of three successive milkings (Spreat and Riggs, 1978). Samples were analyzed by a microbiological cylinder-plate assay and 96-hour sample results were confirmed by radioimmunoassay, with highest results found in samples taken during and twelve hours after the treatment period. The average concentration of gentamicin in the milk samples was <0.02 μg/ml in the 72-hour samples and remained in this range in subsequent samples to 144 hours post-treatment.

Four cows (Holstein, age 4-5 years) each received 200 mg gentamicin (Gentocin) by intramammary infusion in the right front quarter, after which milk was collected at 12-hour intervals to 120 hours post-treatment (Carver et al., 1981). Radioimmunoassay results indicated an average level of 0.08 μg/ml gentamicin at 48 hours, reduced to 0.02 μg/ml at 60 hours and not detectable after 84 hours.

Five normal lactating Holstein cows each received 50 mg of gentamicin sulfate and 100,000 IU of procaine penicillin G by intramammary infusion in each quarter for each of three successive milkings, with a 12 hour spacing between milkings (Spreat and Lobell, 1980 a). Results of the analysis of milk samples from this study (Spreat and Lobell, 1980 b) are given in Table 5. Liver, muscle and fat samples collected from animals slaughtered at 10 and 20 days post-treatment contained no detectable gentamicin residues. Residues in kidneys were 0.57 and 0.81 mg/kg, respectively, in the two animals slaughtered at 20 days, and 0.42, 0.25 and 0.36 mg/kg in the kidneys from the three animals slaughtered 30 days after treatment.

Table 5. Gentamicin residues in milk (mg/l) collected from normal lactating cows following administration of a 10 ml intramammary infusion of Mastogen (50 mg gentamicin sulphate, 100,000 IU procaine penicillin G) per quarter at each of three successive milkings

Hours After Final Treatment	n	Cow 1	Cow 2	Cow 3	Cow 4	Cow 5
12	4	2.30 ± 0.21	2.63 ± 0.96	1.21 ± 0.34	2.84 ± 0.49	1.63 ± 1.08
24	4	0.25 ± 0.02	0.25 ± 0.08	0.26 ± 0.09	0.61 ± 0.19	0.16 ± 0.08
36	4	<0.05	0.06	<0.05	0.10	<0.05
48	4	nd	<0.05	nd	<0.05	<0.05
60	4	nd	nd	nd	nd	<0.05
72	4	nd	nd	nd	nd	nd

nd means not detected; <0.05 means milk from one or more quarters tested positive at or slightly above the assay limit of detection, but the pooled sample for the four quarters should not contain detectable residues.

Four reports were presented on experiments where gentamicin was administered by intrauterine infusion. In each of the first two of these experiments, three mature cows received 200 mg of gentamicin by intrauterine infusion and were slaughtered after withdrawal periods of 15 days (Loy, 1973 a) and 20 days (Loy, 1973 b), respectively, with collection and analysis of kidney, liver, muscle and fat. At 15 days, two of three animals contained detectable gentamicin residues in their kidneys (0.11 and 0.13 mg/kg) (Loy, 1973 a), while at 20 days the kidneys from one of the three animals contained 0.38 mg/kg of gentamicin (Loy, 1973 b). No other tissues collected from the 15 or 20 day animals contained detectable residues of gentamicin, using a microbiological assay.

In a similar study, 5 Holstein cows in early or mid-lactation, 4-5 years of age, received 200 mg of gentamicin by intrauterine infusion and were slaughtered 30 days after treatment (Bickford, 1974). No milk samples collected (6-94 hours post-treatment) contained any detectable gentamicin residues, using a microbiological assay with a limit of detection of 0.01 μg/ml. No detectable residues were found in liver, kidney, muscle or fat samples collected after 30 days withdrawal. The assay limit of detection ranged from 0.04 mg/kg in kidney and fat to 0.16 mg/kg in liver.

Finally, 9 Holstein cows (3-year old) were treated with 200 mg gentamicin by intrauterine infusion and 3 animals were slaughtered at each of 7, 15 and 30 days post-treatment. Milk samples were collected at 12-hour intervals for 5 days (Pavelchak and Lamendola, 1980 b). A tenth Holstein served as a control. All samples were analyzed by radioimmunoassay, with a limit of detection of 0.01 ppm in both milk and tissue samples. No milk samples contained detectable gentamicin residues, nor did muscle, liver or fat samples. Residues in kidney tissues averaged 0.121 mg/kg at day 7, 0.017 mg/kg at day 15 and were only detectable in kidneys from one of three animals at day 30 (0.010 mg/kg). The results of the four experiments were consistent.

Swine

Experiments were conducted using non-radiolabelled gentamicin in the same formulation with healthy (Pavelchak and Lamendola, 1980 c) and colibacillosis-infected (Pavelchak et al., 1980) 3-day-old piglets. Healthy three-day-old piglets which received a single oral dosage of 5 mg of gentamicin (approx. 2.56 mg/kg BW) were sacrificed at 1, 3, 6, 9, 11 and 14 days post-treatment (3-5 piglets per slaughter date) [6]. Average gentamicin residues in kidneys as determined by radioimmunoassay declined from 1.29 mg/kg on day 1 to 0.74 mg/kg at day 6 and 0.04 mg/kg at day 14.

Infected 3-day old piglets treated with a single oral dose of 5 mg of gentamicin (approx. 3.68 mg/kg BW) were slaughtered in groups of three at each of days 1, 3, 6 and 11 post-treatment and kidneys were analyzed by radioimmunoassay (Pavelchak et al., 1980). Average gentamicin residues found in kidneys were: day 1, 0.96 $\pm$ 0.54 mg/kg; day 3, 1.08 $\pm$ 0.70 mg/kg; day 6, 0.35 $\pm$ 0.05 mg/kg; day 11, 0.07 $\pm$ 0.02 mg/kg. The report provided no results for other tissues.

In a related experiment, twenty-six 3-day-old piglets were treated with 5 mg gentamicin orally (estimated dose 3.25 mg/kg BW). After treatments, piglets were assigned randomly into six groups composed by 3 or 5 animals and slaughtered at 1, 3, 6, 9, 11 and 14 days post-treatment. Kidney, liver, muscle, and fat were collected and analyzed by a microbiological assay with a limit of detection of 0.04-0.08 mg/kg, depending on the tissue. No detectable residues were found in any muscle or fat samples, one liver contained 0.4 mg/kg gentamicin at day 3 and residues in kidneys were below the detection limit, 0.08 mg/kg, at day 14. (Lobell et al, 1980) .

Poultry

In 1-day-old chicks treated with 0.2 mg gentamicin by subcutaneous injection (Bickford, 1975), the highest gentamicin residues observed in liver, skin/fat and muscle were in the range of 4.0-4.4 mg/kg within 3 hours of treatment. At 7 days post-treatment, no gentamicin was detectable in muscle, while residues detected in fat/skin and liver were 0.1 and 1.1 mg/kg, respectively.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES AND MILK

General

A radioimmunoassay specific to gentamicin with a claimed limit of detection of 0.02 mg/kg has been described for the analysis of porcine kidneys (Rock and Lamendola, 1978) and tested against an antimicrobial cylinder plate assay and total recovery of radio-labelled gentamicin (Rock et al, 1978 a, b). Results of a direct comparison of these two methods with gentamicin residues as determined by total radioactivity is given in Table 6.

Table 6. Gentamicin residues in kidneys from piglets treated orally with 5 mg gentamicin, assayed by different methods

Days	Gentamicin (mg/kg)		
Withdrawal	Microbiological	RIA	Radiotracer
14	0.61	0.67	0.68
28	0.12	0.20	0.18
35	<0.08	0.07	0.07
42	<0.08	0.05	0.05
49	<0.08	0.02	0.02

In the radioimmunoassay method, tissues were homogenized with 1.0N H_2SO_4, centrifuged and the pellet was re-extracted with water. The combined supernatants were adjusted to pH 7.4 and again centrifuged, after which the supernatant was diluted to 100 ml with Tris buffer and assayed.

Details of a microbiologically based cylinder plate assay system for gentamicin have also been described (Rock et al, 1978 a). Samples were prepared for assay using extraction procedures similar to those described above. Petri dishes were prepared with a pre-seeded agar medium containing an 0.1% suspension of *S. epidermidis ATCC 12228* over an unseeded base layer. Assay dishes stood at room temperature for one-half hour prior to sample addition. Six stainless steel cylinders were then placed on the surface of the agar and three were alternately filled with standard or test samples, while the remainder were filled with reference solution. After 1 h pre-incubation at room temperature, the assay dishes were incubated overnight at 35°. Concentrations were estimated from the diameters of the zones of growth inhibition. The test has a claimed limit of detection of 0.08 mg/kg for gentamicin residues in porcine kidney. Details of the selectivity of this test against other antibiotics were not provided.

Other assay methods for gentamicin residues in animal-derived foods that have been described include thin layer chromatography (TLC) (Lagner and Teufel, 1972) and TLC-bioautography (Yoshimura et al., 1982; Salisbury et al., 1989). None of these TLC-based methods appear sufficiently sensitive for routine regulatory use. Extraction methods for quantitation of gentamicin residues in kidney and muscle using a commercially available ELISA have been reported, with the additional application of the method to gentamicin in milk (Brown et al., 1990). Among the gentamicin-specific test kit technologies that are commercially available are receptor assays and a variety of kits using ELISA technology in well or card formats. While independent testing has not been done on all these tests in all matrices of interest, there does appear to be a selection of commercially available test kit technologies suitable for use in regulatory screening programs for gentamicin.

The determination of gentamicin in kidney using ion-pairing liquid chromatography (LC) followed by post-column derivatization with o-phthalaldehyde (OPA) has been reported, but no detection limit was given (Lacharte et al., 1983). A similar approach with pre-column derivatization with OPA for the determination of gentamicin in muscle, with a detection limit of 0.2 mg/kg and recoveries of 69-105% has also been described (Agarwal, 1989). An LC method for the qualitative identification of gentamicin residues in porcine tissue at levels above 0.4 mg/kg has also been identified (FSIS, 1991). It is not clear that any of these methods are suitable for routine use in a regulatory laboratory.

More recently, methodology for the quantitative determination of gentamicin residues in muscle, liver, kidney and fat from swine, sheep and cattle has been included in a publication prepared on behalf of the Commission of the European Communities (Heitzman, 1994). This methodology references an earlier publication (Sar et al., 1993). The principal steps of the method include homogenization, protein precipitation using 5% trichloroacetic acid, clean up on an ion exchange column using CM-Sephadex, then high performance liquid chromatography on an end-capped 5μ RP-18 column at 45°, followed by post-column derivatization with OPA. The method has a claimed limit of quantitation of 0.05 mg/kg for fat and muscle and 0.10 mg/kg for liver and kidney, with reported recoveries in muscle of 74% at 0.8 mg/kg and 88% at 0.1 mg/kg. Recoveries are in the same range for other tissues.

No data have been published on inter-laboratory trials.

A multi-residue confirmation method for aminoglycoside antibiotics in bovine kidney using ion spray LC/MS/MS, applicable to four components of gentamicin (C_1, C_{1a}, C_2 and C_{2a}), has recently been reported (McLaughlin et al., 1994). The method includes clean-up by matrix solid phase dispersion, followed by separation using a gradient LC system. Three ions were monitored for each of the gentamicin components and the method was tested successfully on incurred samples. Gentamicin components were recovered at about 60% and minimum detectable levels for each of the four components were <0.10 mg/kg. The method appears suitable as a confirmatory procedure for laboratories which have the required equipment.

APPRAISAL

Studies in several species indicated that there is little absorption of gentamicin into edible tissues, other than kidney, for most forms of drug administration. However, little information was available concerning the persistence of gentamicin residues at injection sites (2 studies, 2 and 5 samples, respectively). Radiolabeled residue depletion studies were conducted only in piglets. These and other residue depletion studies done with swine and cattle showed that residues were most persistent from intramuscular injection and this method of administration also produced persistent detectable levels in liver. No significant persistent residues of gentamicin were found in muscle samples in any of the studies conducted. Residues also cleared from milk within a week of administration of gentamicin by intramammary infusion or intramuscular injection. Treatment of dairy cattle by interauterine infusion produced no detectable gentamicin residues in milk at any subsequent milkings, but residues were detectable in kidneys for up to 30 days post-treatment. Based on these studies, the kidney is the appropriate target tissue for residue monitoring. The studies also showed little indication of metabolism based on the close agreement of results found by three different assay methods in several studies, so gentamicin parent compound(s) should be the appropriate marker residue. It was noted that there are three or four significant components in gentamicin which should be detected in a determinative or confirmatory analytical method.

The single study reported for poultry in which 1-day-old chicks were injected subcutaneously with gentamicin was insufficient to assess the potential for persistence of gentamicin in market weight broiler chickens or in eggs which might result from this treatment. Residues in excess of 1 mg/kg were detectable in kidney and liver samples at 7 days post-treatment.

The status of current residue methodology, in brief, is that there are available commercial test kit technologies suitable for the screening of milk and tissue samples for gentamicin residues and assays based on microbial growth inhibition are available for laboratory determination of these residues. There is, however, a need for a validated chemical assay for the determination of gentamicin residues in milk. While no multi-laboratory method validations have been reported, one recently reported LC method meets the performance criteria of the Codex Committee on Residues of Veterinary Drugs in Foods.. A recently published LC/MS/MS method should be suitable for confirmation of gentamicin residues in tissue samples, but the equipment is not currently routinely available in all regulatory laboratories. Based on currently published methodologies, limits of quantitation and confirmation in the range of 0.1 mg/kg appear feasible.

Maximum Residue Limits

Based on the temporary ADI of 0-4 μg/kg body weight established by the Committee using a microbiological

end point, the permitted daily intake of gentamicin would be 240 μg of antimicrobially active gentamicin residues contributed by 500 g of food-animal tissues together with 1.5 l of milk in the diet of a 60-kg person. This was expressed as parent drug as there was no indication of significant metabolism. The Committee recommended temporary MRLs for gentamicin of 100 μg/kg for muscle and fat, 200 μg/kg for liver, and 1000 μg/kg for kidney in both cattle and pigs, as well as 100 μg/l for cattle milk, expressed as parent drug.

The temporary MRL allocated to milk takes into account the limit of quantification of current analytical methods. No MRLs were assigned to poultry or eggs because appropriate data were not available. The temporary MRLs recommended above would result in a daily maximum intake of 255 μg of gentamicin residues based on a daily intake of 300 g of muscle, 100 g of liver, 50 g of kidney, 50 g of fat, and 1.5 l of milk.

The following information is required for evaluation in 1997:

- a validated chemical analytical method with a limit of quantification at or preferably below the MRL recommended for milk.

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