The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines and Inspections

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

GENTAMICIN

SUMMARY REPORT (3)

1. Gentamicin is a complex mixture, the main components being gentamicin C1, gentamicin C1a, gentamicin C2 and gentamicin C2a. Gentamicin is an aminoglycoside antibiotic indicated for the treatment of a variety of bacterial infections in pigs and cattle. In veterinary medicine is normally used as the sulphate salt. The recommended dose regimen is about 4 mg/kg bw for cattle and pigs once or twice daily for 3 to 5 days either by parenteral administration or by oral administration.

   Currently, gentamicin is included in Annex III of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Gentamicin	Gentamicin	Bovine, porcine	50 µg/kg 50 µg/kg 200 µg/kg 750 µg/kg	Muscle Fat Liver Kidney	Provisional MRLs expire on 1.6.2002
		Bovine	100 µg/kg	Milk	

   Additional data were provided in response to the list of questions, further to the establishment of provisional MRLs for gentamicin.

2. In humans, gentamicin is generally administered intramuscularly every 8 hours to provide a total daily dose of 3 mg/kg bw/day. It may be used as eye collyrium or ophthalmic ointment.

3. Only a very limited number of studies on the pharmacokinetics of gentamicin were available, however, the aminoglycosides have been extensively reviewed. In view of their polar nature and high aqueous solubility, aminoglycosides are poorly absorbed after oral administration**.** However**,** the absorption after intramuscular or subcutaneous administration in most species is good with peak blood levels occurring within 30 to 90 minutes after an intramuscular injection. It is recognised that aminoglycosides are not metabolised and are eliminated unchanged in the urine by glomerular filtration. Within 24 hours, 80% to 90% of the administered dose is eliminated.

4. Gentamicin has low acute toxicity after oral administration in rodents (LD_{50} values 8000 to 1 000 mg/kg bw) but is more toxic following intravenous (LD_{50} values 37 to 67 mg/kg bw) and intramuscular (LD_{50} values 200 to 890 mg/kg bw) administration, again suggesting poor absorption after oral administration.

5. Gentamicin has been extensively studied in subchronic toxicity tests, after intramuscular and subcutaneous administration to rats and dogs for periods of up to 12 months.

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In rats given intramuscular injections of gentamicin at doses of 25, 50 and 200 mg/kg bw/day, 6 days per week for 4 weeks, the kidney was the primary target organ with interstitial nephritis and toxic nephrosis as the main findings. The NOEL derived from this study was 25 mg/kg bw/day.

After subcutaneous administration for up to 28 days with doses of 50 and 150 mg/kg bw/day, the kidney was again the target organ. Altered renal function was noted at the lowest dose used. No NOEL could be retained from this study.

In an oral study, rats were fed diets containing gentamicin to maintain doses of 4, 19 and 116 mg/kg bw/day for a period of 3 months. The only adverse signs noted were soft stools in the group administered 116 mg/kg bw/day. .The NOEL was 19 mg/kg bw/day.

6. In dogs given oral daily doses of gentamicin sulphate at doses of 0, 2, 10, 60 and 120 mg/kg bw/day for up to 14 weeks, the main effect was renal toxicity. The NOEL was 10 mg/kg bw/day.

   In a one year study, the animals received gentamicin by the intramuscular route at doses of 0, 3, 5 and 8 mg/kg bw/day, 5 days per week. Pale renal cortices and interstitial nephritis were seen in all treatment groups with a dose-response relationship. No NOEL was identified.

7. In a special study groups of 3 female monkeys were injected intramuscularly with doses of 0, 25, 50 mg/kg bw/day for 35 days. Hair cell loss and a reduction in the thickness of sensory epithelium were observed at the high dose. In the organ of Corti, cochlear hair cell loss was noted at 50 mg/kg. A NOEL of 25 mg/kg bw was derived from this study.

8. A multigeneration study in the rat showed no adverse effects on reproduction after intramuscular injections of 5 and 20 mg/kg bw/day.

9. Several teratogenicity studies were carried out in mice, rats, guinea pigs and rabbits.

   After subcutaneous administration of gentamicin by the subcutaneous route to mice at doses of 0, 1, 10 and 100 mg/kg bw/day during gestation days 6 to 10, foetotoxicity was reported at 10 and 100 mg/kg bw/day. No evidence of teratogenic effects was reported.

   After intramuscular administrations of gentamicin to rats at doses of 0, 25 and 50 mg/kg bw/day, 6 days per week, only foetotoxicity was reported at the highest dosage. No teratogenic effects were reported.

   In guinea-pigs, intramuscular doses of 4 mg/kg bw/day given on gestation days 48 to 54 did not induce teratogenic effects.

   In rabbits after intramuscular administrations of gentamicin at doses of 0.8 and 4 mg/kg bw/day on gestation days 6 to 16, no teratogenic effects were reported.

   As the design of these studies was not in accordance with the current requirements no NOELs could be established.

10. Gentamicin has been tested in a range of *in vitro* genotoxicity studies (*Salmonella* microsomal assay, test for mitotic crossing over, gene conversion, DNA repair, Rec-assay) and most gave negative results. However, positive results were noted in *in vitro* tests for forward mutation in *Escherichia coli* at a cytotoxic dose level, in a test for chromosome aberrations in mouse L-cells and in a test for sister chromatid exchange in human fibroblasts. The design of these studies was inadequate to evaluate the genotoxic potential of gentamicin.

    Three GLP-compliant mutagenicity studies have been carried out with gentamicin, tested as the sulphate salt.

    Gentamicin was examined for cytotoxicity and mutagenicity in a chromosomal aberration assay in CKO-K1 cells in the absence and presence of metabolic activation. As a result of the cytotoxicity tests, mutagenic activity was tested using final concentrations of 5000, 2000 and 800 µg/ml. Both positive and negative controls were included. Gentamicin sulphate was negative for inducing chromosomal aberrations in Chinese hamster ovary (CHO) cells, both in presence and absence of metabolic activation.

In the second study gentamicin sulphate was tested for mutagenic activity in the CHO/HGPRT gene mutation assay. The mutagenic activity was tested using final doses of 5000, 2000, 800, 320 and 128 µg/ml in the presence and absence of a metabolic activation system. Both negative and positive controls were included in the study. Gentamicin sulphate was weakly or non-toxic to Chinese hamster ovary cells at concentrations ranging from 8 to 5000 µg/ml, where cell viability was lower than 50% at 2000 and 5000 µg/ml. This effect was not dose-related and was not reproducible in the second assay. Gentamicin sulphate was negative for inducing a mutagenic response in the CHO/HGPRT gene mutation assay in the presence or in the absence of metabolic activation.

The mutagenic potential of gentamicin sulphate *in vivo* was investigated in a micronucleus test in bone marrow erythrocytes of CD-1 mice. Because of the low absorption of gentamicin after oral administration, the intravenous route was used to ensure exposure of the target bone marrow cells. The mice were dosed at either 20, 40 and 80 mg/kg. No micronucleus induction was detected in bone marrow erythrocytes of any mice treated with the three concentrations of gentamicin. Negative control animals showed normal backgrounds levels of micronuclei and the positive controls animals (cyclophosphamide), had substantial increases in the number of bone marrow micronuclei when killed and sampled 24 hours post treatment. Gentamicin sulphate was negative for inducing micronuclei in bone marrow cells of CD-1 mice when tested to the maximum tolerated intravenous dose of 80 mg/kg.

Although gentamicin gave positive results in some old inadequate *in vitro* mutagenicity tests, these findings could not be confirmed in a battery of well conducted genotoxicity tests (two *in vitro* tests (chromosomal aberration assay in CHO-K1 cells, a CHO/HGPRT gene mutation assay) and one *in vivo* mouse micronucleus test.

Overall it was concluded that gentamicin is unlikely to be genotoxic.

11. No carcinogenicity studies were available.

    A report on possible structural similarities of gentamicin and known carcinogens has been compiled. The structural features of gentamicin were compared to those in two lists of structural features that are considered to indicate an increased probability of carcinogenic activity in animals. None of the structural features listed in the two compilations were present in gentamicin.

    In addition the Joint WHO/FAO Expert Committee on Food Additives (JECFA) in its evaluation assumed that as no carcinogenic effect has been observed with the other aminoglycosides that have been tested in long-term bioassays in animals, a possible carcinogenic activity of gentamicin is unlikely.

12. In humans, toxic findings such as nephrotoxicity and ototoxicity have been reported.

    Nephrotoxicity has been reported in renally compromised patients and in patients treated for longer periods at the therapeutic dosage. Although extensively studied, the mechanism of gentamicin-induced renal toxicity is incompletely understood.

    Ototoxicity in patients treated with gentamicin occurred after intravenous or intramuscular administration of the drug, primarily in patients with renal impairment. Gentamicin accumulates at relatively high concentrations in the endolymph and perilymph of the inner ear, leading to the primary cause of ototoxicity, the degeneration of vestibular sensory cells. It has been reported that ototoxicity from gentamicin is related to concentrations in blood that exceeds 10 µg/ml. Maintaining levels below this limit will generally protect patients from the development of these effects.

    There are no known reports of its use in humans being associated with reproductive toxicity or teratogenicity.

    As gentamicin is poorly absorbed, the risk of nephrotoxicity and ototoxicity resulting from the ingestion of residues of residues in foodstuffs was considered as negligible.

13. A toxicological ADI of 100 µg/kg bw was calculated by applying a safety factor of 100 to the NOEL of 10 mg/kg bw/day which was established in the 14-week toxicity study in dog.

14. Three GLP studies were provided on the effects of gentamicin on micro-organisms used in the food industry.

    The MIC of gentamicin against 15 pure starter cultures isolated from mixed commercial starter cultures was determined in the presence and absence of milk using a broth microdilution method. There was no inhibiting effect on growth of any starter culture at a gentamicin concentration of 62 µg/l. The presence of milk did not significantly affect the gentamicin sensitivity of 8 strains.

    The MIC of gentamicin against 6 mixed starter cultures used in the food industry was determined using a broth microdilution method in the presence and absence of milk. There was no inhibitory effect on the growth of any mixed culture at a gentamicin concentration of 500 µg/l. The presence of milk did not significantly affect the gentamicin sensitivity of 3 mixed cultures.

    The effect of gentamicin was determined on the acidification performance of 3 commercial dairy starter cultures containing mixed bacterial strains. Gentamicin concentrations of 10, 100 and 500 µg/l were tested. Gentamicin concentrations of 10 and 100 µg/l did not inhibit acid production significantly and would not have any detrimental impact on an industrial process.

    The concentration of 100 µg/l was retained as the concentration without effects on dairy starter cultures.

15. The effect of gentamicin on the growth of 110 bacterial strains obtained from the human gastro-intestinal flora was evaluated after incubation *in vitro*. MIC values were determined for 80 isolates of the 8 predominant groups of anaerobic microflora in the human intestine. These included *Bacteroides spp*., *Lactobacillus spp*., *Bifidobacterium spp*., *Prevotella spp*., *Eubacterium spp*., *Clostridium spp*., *Fusobacterium spp*. and anaerobic Gram positive cocci. In addition data were provided for 30 isolates of the facultative anaerobes *Enterococcus spp., Proteus spp.* and coliforms. Geometric MIC values ranged from 0.04 µg/ml for *Proteus* to greater than 128 µg/ml for *Prevotella* and bacteroides species. The overall geometric mean MIC of gentamicin for all genera tested was 8.86 µg/ml and the 90 % confidence limit of the geometric mean MIC_{50} was estimated at 1.5 µg/ml and will be retained for the calculation of the microbiological ADI.

    For the assessment of the microbiological risk, use was made of the formula recommended by the CVMP:

$$\text{ADI (µg/kg bw)} = \frac{\dfrac{\text{geometric mean } MIC_{50} \times CF2}{CF1} \text{ (µg/ml)} \times \text{daily faecal bolus (150 ml)}}{\text{Fraction of an oral dose Available for micro-organisms} \times \text{weight of human (60 kg)}}$$

    Based on the above formula, the microbiological ADI can be calculated as follows:

$$\text{ADI} = \frac{\dfrac{1.5 \times 1}{1} \times 150}{1 \times 60} = 4 \text{ µg/kg bw i.e.} = 240 \text{ µg/person}$$

    The following assumptions were made:

    - CF1 = 1 because the 90% confidence limit geometric MIC_{50} value for all tested strains was used;
    - CF2 = 1 because no data were available to correct for extrapolation from the *in vitro* to the *in vivo* situation;
    - 150 g was the weight of the daily faecal bolus;
    - 1 = the fraction of the oral dose available to the micro-organisms at the distal part of the gastrointestinal tract.

16. At its fiftieth meeting, JECFA established a definitive microbiological ADI for gentamicin of 22 µg/kg bw i.e. 1320 µg/person based on the geometric MIC_{50} of the most sensitive relevant

genera isolated from the human gastrointestinal tract, *Eubacterium spp,*. 6 µg/ml at an inoculum density of 10^6 CFU.

The CVMP could not follow this approach as the CVMP formula is based either on the geometric mean or on the MIC_{50} of the most sensitive strain. JECFA recognised that *Enterococci*, *Coliforms* and *Proteus* were the sensitive strains, JECFA based its calculation on the most sensitive relevant genera, *Eubacterium* spp.

17. The microbiological ADI is lower than the toxicological ADI and therefore it was considered to be the most relevant ADI for assessing the risk to consumers.

18. Several radiolabelled studies combining different analytical methods (microbiological and radioimmunoassay methods) were carried out in pigs.

    Groups of 3 six-week old pigs received ^{3}H-gentamicin sulphate doses corresponding approximately to 12 mg/kg bw/day for 3 days via drinking water. Animals were slaughtered at 1, 3, 5 and 7 days post medication. For muscle and fat, the mean total radioactivity levels was below the limit of quantification (30 µg equivalents gentamicin/kg) at all sampling times. The levels of radioactivity in liver and kidney were 112 µg/kg and 180 µg equivalents gentamicin/kg, one day after treatment and declined to 76 and 62 µg/kg at 3 days, respectively. At later sampling, they were in the magnitude of 40 µg/kg in both tissues.

    In addition, microbiological assay and radioimmunoassay also determined the concentrations of gentamicin residues in kidney. At one day after treatment, the mean concentration of microbiologically active residues was 210 µg microbiologically active residues expressed as gentamicin/kg and 160 µg/kg gentamicin when determined by radioimmunoassay. At later samplings, the concentrations of residues with antimicrobial activity were below the limit of quantification (lower than 80 µg/kg) whereas the gentamicin concentrations by radioimmunoassay were 40 and less than 30 µg/kg at 3 and 7 days after treatment. At one day after treatment, the ratio of gentamicin with regard to the total residues with antimicrobial activity was 0.76 in kidney. At later sampling the concentrations were too low to determine the ratios. No attempt was made to determine the ratios in other edible tissues.

    In another study, groups of 3 or 4 three-day old piglets were treated with a single oral dose of 5 mg of ^{3}H-gentamicin. Animals were slaughtered at 1, 3, 6, 11, 14 and 17 (2 animals only in this group) days after treatment. At 1 day after administration, the total radioactivity concentrations in fat and muscle were in the magnitude of the limit of quantification of 50 µg/kg whereas the mean levels of total radioactivity were 4900 and 210 µg equivalents gentamicin/kg in liver and kidney, respectively. At day 3, the radioactivity levels were in the magnitude of 50 µg equivalents gentamicin/kg in liver and 450 µg/kg in kidney. At day 6, 270 µg equivalents gentamicin/kg were measured in kidney and 69 µg/kg in liver. At later sampling times, the radioactivity levels were below the limit of quantification 50 µg/kg in kidney and liver.

    In another study, groups of 3-day old piglets were treated with a single intramuscular dose of 5 mg of ^{3}H-gentamicin. Animals were slaughtered at 14 (2 animals), 28 (3 animals), 35 (3 animals), 42 (3 animals) and 49 (1 animal) days after treatment. At 14 days after administration, the total radioactivity concentrations in fat and muscle were in the magnitude of the limit of quantification of 20 µg/kg whereas the mean levels of total radioactivity were 117, 419 and 677 µg equivalents gentamicin at the injection site, in liver and kidney, respectively. At later sampling time, the radioactivity could only be measured in liver and kidney: at day 28, 111 and 178 µg equivalents gentamicin/kg; at day 35: 60 and 73 µg/kg; at day 42: 37 and 51 µg/kg; at day 49: 24 and 22 µg/kg, respectively. Gentamicin residues in kidney were also determined by a microbiological assay, radioimmunoassay. At 14 days after treatment, the mean concentration of antimicrobiologically active residues was 610 µg antimicrobiologically active residues expressed as gentamicin/kg and that of gentamicin 672 µg/kg. At day 28, they were 123 µg/kg and 200 µg/kg, respectively. At later sampling time, the concentrations of residues with antimicrobial activity were below the limit of quantification (lower than 80 µg/kg) whereas the gentamicin concentrations were 71, 46 and 20 µg/kg at day 35, 42 and 49, respectively.

At day 14, as the concentrations of gentamicin determined by radioimmunoassay were higher than that measured by microbiology, no conclusion could be given on the ratio of gentamicin with regard to the total antimicrobiologically active residues. No information on the ratio for the other edible tissue was provided.

From the information provided, it was not possible to establish the ratio of gentamicin with regard to the total antimicrobiologically active residues in edible tissues of porcine. As whatever the analytical method used (radiometric, microbiological test and immunoassay), the concentrations of gentamicin in muscle and fat were always below the limit of quantification, such information was considered not necessary.

19. Several non-radiolabelled residue depletion studies were performed in neonatal piglets and young pigs.

In one study, groups of 5 three-day old piglets were treated with a single oral dose of 5 mg of gentamicin. Animals were slaughtered at 1, 3, 6, 11 and 14 days post medication. Gentamicin residues in edible tissues were determined by a microbiological assay. At all sampling times, the antimicrobiologically active residues were below the limit of quantification in muscle and liver (less than 80 µg/kg) and in fat (less than 40 µg/kg). Only significant amounts of antimicrobiologically active compounds were measured in kidney: 1066, 630, 610, 175, 152, less than 80 µg antimicrobiologically active residues/kg at 1, 3, 6, 9, 11 and 14 days after treatment.

In a second study, groups of 5 three-day-old piglets were treated with a single oral dose of 5 mg of gentamicin. Animals were slaughtered at 1, 3, 6, 11 and 14 days after treatment. Gentamicin residues were determined by a microbiological assay. Significant amounts of antimicrobiologically active compounds were measured in kidney: 1078, 1028, 291, 394, 151, 42 µg antimicrobiologically active residues/kg at 1, 3, 6, 9, 11 and 14 days after treatment.

In a third study, groups of 4 three-day old piglets were treated with a single intramuscular dose of 5 mg of gentamicin. Animals were slaughtered at 2, 3, 4, 5, 6, 7, 8 and 9 weeks post medication. Gentamicin residues in edible tissues were determined by a microbiological assay. At all sampling times, the residues with antimicrobial activity were below the limit of quantification in muscle and at the injection site (less than 100 µg/kg) and in fat (less than 40 µg/kg), Only significant amounts of residues with antimicrobial activity were measured in kidney: 470, 340, 250, less than 150 µg microbiologically active residues/kg at 2, 3, 4, 5 weeks. At later sampling times, the concentrations were less than 90 µg antimicrobiologically active residues/kg. Significant amounts of residues with antimicrobial activity were also measured in liver: 320, 130 µg antimicrobiologically active residues/kg at 2 and 3 weeks after treatment. At later sampling times, the concentrations were less than 100 µg antimicrobiologically active residues/kg.

In a fourth study neonatal piglets were treated by the oral route with 5 mg gentamicin, daily for 3 days (equivalent to a mean dose level of about 3.7 mg/kg). Groups of 4 animals were sacrificed 13 and 29 days after the final administration. Gentamicin concentrations were determined in muscle, liver and kidney using a fluorescence polarisation immunoassay**,** the limit of quantification being 50 µg/kg for muscle, 250 µg/kg for liver and 500 µg/kg for kidney. The concentrations of gentamicin in kidney and liver samples were also analysed using a microbiological assay. At 13 days after the last administration, gentamicin could not be detected in muscle (lower than 50 µg/kg, the limit of detection). In liver, the concentrations of gentamicin were below the limits of quantification of the microbiological and immunoassay analytical methods (lower than 500 and 250 µg/kg respectively). In kidney, the concentrations of gentamicin were below the limits of quantification of the microbiological (250 µg/kg) and a mean value of 265 µg/kg was measured by the immunoassay method. At 29 days, in all edible tissues, gentamicin could not be quantified or even detected.

Groups of 4 pigs were treated by the intramuscular route at a dose level of approximately 4 mg gentamicin/kg bw/day for 3 consecutive days. Samples of muscle, liver, kidney, fat and injection site were analysed by HPLC with fluorescence detection, the limit of quantification being (200 µg/kg for liver, 1000 µg/kg for kidney, 100 µg/kg for muscle and fat). Animals were sacrificed at 10, 20, 30, 40, 50, 60 and 70 days after treatment. The concentrations of gentamicin C2a (component G4) in muscle were below the limit of quantification at every sampling time. The mean residue concentrations at the injection site were 204 µg/kg at day 10 and below the 100 µg/kg for the other sampling times. The mean residue concentrations of gentamicin C2 (component G3) in fat were below the limit of quantification for each sampling time with the exception of day 30 when a mean value of 160 µg/kg was measured. The mean residue concentrations of gentamicin C2 in kidney (component G3) were 12746 µg/kg at day 10, 5094 µg/kg at day 20, 1170 µg/kg at day 30, below the limit of quantification at 40, 50 and 60 days. The mean residue concentration of gentamicin C2a (component G4) in liver at day 10, 1142 µg/kg at day 20, 685 µg/kg at day 30, 394 µg/kg at day 40, 288 µg/kg at day 50 and below 200 µg/kg at day 60 and 70.

20. No radiolabelled studies were provided in bovine. However such studies were not requested as the relevant acceptable daily intake was based on a microbiological end-point.

    In one non-radiolabelled study, groups of 5 to 3 calves weighing approximately 60 kg bw were treated with repeated intramuscular doses of 4 mg of gentamicin per day for 3 days. Animals were slaughtered at 7, 30, 60, 70 and 80 days after the last injection. Gentamicin residues in edible tissues were determined by a microbiological assay using *Staphylococcus epidermidis* as test organism, the limit of quantification being 50 µg/kg. At all sampling times, the antimicrobiologically active residues were below the limit of quantification in muscle. Significant amounts of antimicrobiologically active compounds were measured in liver and kidney: 3600 and more than 10 000 µg antimicrobiologically active residues/kg at day 7, 800 and 2000 µg/kg at day 30, 500 and 1100 µg/kg at day 60, 300 and 900 µg/kg at day 70 and 30 and 600 µg/kg at day 80, respectively.

    Groups of 4 calves received gentamicin by intramuscular route at a dose level of approximately 4 mg gentamicin/kg bw for 3 consecutive days. Animals were sacrificed 10, 20, 30, 40, 50, 60, 70 and 80 days after the last administration. Residues of gentamicin in muscle, liver, kidney, fat and the injection site were analysed by HPLC with fluorescence detection, the limit of quantification being 200 µg/kg for liver, 1000 µg/kg for kidney, 100 µg/kg for muscle and fat. The mean residue concentrations of gentamicin C2a (component G4) in liver were 2784 µg/kg at day 10, 1462 µg/kg at day 20, 726 µg/kg at day 30, 809 µg/kg at day 40, 404 µg/kg at day 50, 612 µg/kg at day 60, lower than 200 µg/kg at day 70 and 208 µg/kg at day 80. The mean residue concentrations of gentamicin C1a (component G2) in kidney were 20758 µg/kg at day 10, 2121 µg/kg at day 20, 2689 µg/kg at day 30, 1315 µg/kg at day 40 and 1261 µg/kg at day 50, below 1000 µg/kg at day 60, 70 and 80. Gentamicin C2 (component G3) residues in muscle and fat were always below 100 µg/kg, the limit of quantification of the analytical method. The mean residue concentrations at the injection site samples were in the magnitude of 200 µg/kg up to 70 days after treatment. At 80 days, they were below the limit of quantification (100 µg/kg).

    Several depletion studies were carried out using intramammary or intrauterine administration of gentamicin sulphate at different dosages. Significant amounts of antimicrobiologically active residues could be measured only in kidney samples taken up to 20 days after the end of treatment.

21. Several cold studies were provided to follow the depletion of gentamicin in bovine milk.

    In one study, five lactating cows were treated with repeated intramuscular doses of 4 mg gentamicin/kg bw/day for 3 days. Milk samples were collected up to 90 hours after treatment. Gentamicin residues in edible tissues were determined by a microbiological assay using *Staphylococcus epidermis* as test organism, the limit of quantification being 50 µg/kg. No antimicrobiologically active residues could be detected in any milk sample collected.

In a second study, 5 lactating Holstein cows were treated with three successive infusions of 10 ml containing 100 mg gentamicin sulphate and 100 000 units of procaine penicillin per quarter. Samples of milk were taken from the total production of each quarter at 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132 and 144 hours following the third injection. The concentrations of gentamicin were analysed by a microbiological assay using *Staphylococcus epidermidus* with a sensitivity of 10 µg/kg. The mean concentrations of gentamicin were 19250 µg/l at 12 hours after treatment, then they declined to 1910, 330, 80, 40, to 20 and to 10 µg antimicrobiologically active residues expressed as gentamicin equivalents/kg at 24, 36, 48, 60, 72 and 132 hours after treatment.

In a third study, five lactating Holstein cows were treated with three successive infusions of 10 ml containing 50 mg gentamicin sulphate and 100 000 units of procaine penicillin per quarter. Samples of milk were taken from the total production of each quarter at 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132 and 144 hours following the third injection. The concentrations of gentamicin were analysed by a microbiological assay using *Staphylococcus epidermidus* with a sensitivity of: 40 µg/kg. The highest concentration of gentamicin was 2500 µg/l at 12 hours after treatment, then declined to 148 µ/kg, and to 40 to 100 µg/kg at 24, 36 hours after treatment, respectively.

22. No information was provided on the ratio of gentamicin with regard to the total antimicrobiologically active residues in most of the edible tissues of the target tissue including milk. However, having considered that this substance belongs to the aminoglycosides, which are not metabolised to any extent and are excreted unchanged *via* the kidney, such information was not considered as necessary.The parent compound was assumed to represent all the relevant antimicrobiologically active residues. The marker residue was retained as the sum of gentamicin C1, C1a, C2 and C2a, which corresponds to more than 90% of the antimicrobial activity of gentamicin.

23. Based on a microbiological ADI of 22 µg/kg bw i.e. 1320 µg/person the JECFA established the following MRLs for gentamicin in bovine and porcine species: 100 µg/kg for muscle and fat, 2000 µg/kg for liver, 5000 µg/kg for kidney and 200 µg/l for bovine milk. The CVMP could not follow this approach because the CVMP had agreed a lower ADI. In addition, the CVMP had considered new information which lead to a different definition of the marker residue.

24. Analytical methods, using liquid chromatography with mass spectrometric detection (LC/MS) were available for the determination of gentamicin in bovine and porcine tissues and in bovine milk . Molecular ions for the components of the marker residue were individually detected in positive ion mode with unit mass resolution to a common 322.1 m/z transition. The methods were well described according to ISO 78/2 and were fully validated according to Volume VI of the Rules Governing Medicinal Products in the European Community. The limits of quantification in bovine and porcine species were 100 µg/kg for liver, 25 µg/kg for muscle and for fat, 375 µg/kg for kidney and 50 µg/kg for bovine milk.

Conclusions and recommendation

Having considered that:

- a conservative microbiological ADI of 4 µg/kg bw i.e. 240 µg/person was established;
- a lower ADI than the one set by JECFA was established by the CVMP, therefore JECFA MRLs could not be accommodated;
- the marker residue was retained as the sum of gentamicin C1, C1a, C2 and C2a which corresponds to more than 90% of the antimicrobial activity of gentamicin.
- validated analytical methods were available for monitoring residues of gentamicin in bovine and porcine tissues and in bovine milk

the Committee for Veterinary Medicinal Products recommends the inclusion of gentamicin in Annex I of Council Regulation (EEC) N° 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Gentamicin	Sum of gentamicin C1, gentamicin C1a, gentamicin C2 and gentamicin C2a	Bovine,	50 µg/kg 50 µg/kg 200 µg/kg 750 µg/kg 100 µg/kg	Muscle Fat Liver Kidney Milk	
		Porcine	50 µg/kg 50 µg/kg 200 µg/kg 750 µg/kg	Muscle Skin + Fat Liver Kidney	

Based on these MRLs, the daily intake represents 94% of the microbiological ADI.