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APRAMYCIN page 39-63

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APRAMYCIN

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1. EXPLANATION

Apramycin (Chemical Abstracts Service No. 65710-07-8) is a broad-spectrum aminocyclitol antibiotic produced by a strain of *Streptomyces tenebrarius*. It is effective against both Gram-positive and Gram-negative bacteria, including isolates of *Escherichia coli* and *Salmonella* species, and some strains of mycoplasma from farm animals and human origin. Apramycin is extracted from the fermentation medium as apramycin sulfate at a purity of at least 85%, and activity is expressed as equivalents of apramycin base using a microbiological assay.

Apramycin exerts its antibacterial effect by inhibiting protein synthesis at the level of peptidyl translocation. It is used for the treatment of a variety of enteropathogenic infectious diseases in target species such as cattle, swine, poultry and rabbits (e.g. colibacillosis, salmonellosis and other bacterial infections in calves and pigs, *Escherichia coli* septicaemia, colibacillosis, salmonellosis and other bacterial infections in poultry and bacterial enteritis including colibacillosis in rabbits). Apramycin is marketed as the sulfate in soluble powder (to be incorporated in drinking-water) and premix formulations (to be incorporated in feed). Apramycin has also been used as an intramuscular injection in calves and in an oral dosage form in neonatal lambs, calves and pigs. Apramycin is not used in human medicine.

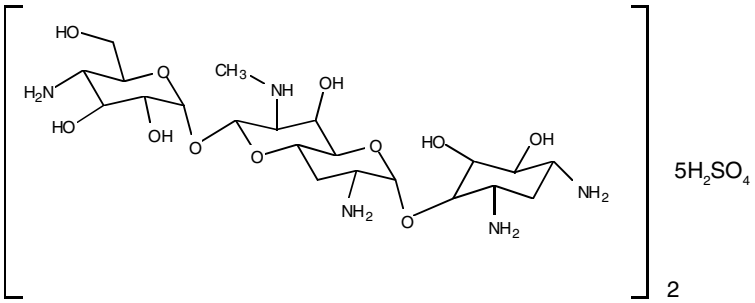
The recommended doses are 20–40 mg/kg body weight (bw) per day of soluble power incorporated in drinking-water or milk replacer or of premix incorporated in feed for calves for 5 days; 7.5–12.5 mg/kg bw per day of soluble power incorporated in drinking-water for 7 days or 4–8 mg/kg bw per day of premix incorporated in feed for up to 28 days in pigs; 20–80 mg/kg bw per day of soluble power incorporated in drinking-water for 5–7 days or 2–5 mg/kg bw per day of premix incorporated in feed for 5 days in poultry; and 10–15 mg/kg bw per day of soluble powder incorporated in drinking-water for 5–8 days or 5–10 mg/kg bw per day of premix incorporated in feed for up to 21 days in rabbits.

Apramycin has not been previously evaluated by the Committee. The Committee evaluated apramycin to establish an acceptable daily intake (ADI) and recommend maximum residue limits (MRLs) in cattle, pig and chicken tissues at the request of the Nineteenth Session of the Codex Committee on Residues of Veterinary Drugs in Foods (FAO/WHO, 2011).

In addition to a submission from the sponsor, a literature search was conducted using Toxnet. Only one further relevant study report was identified in the published literature.

The structure of apramycin ((2*R*,3*R*,4*R*,5*S*,6*R*)-5-amino-2-[((1*R*,2*R*,3*R*,4*R*,6*R*,8*R*)-8-amino-9-[(1*R*,2*S*,3*R*,4*R*,6*R*)-4,6-diamino-2,3-dihydroxy-cyclohexyl]-oxy-2-hydroxy-3-methylamino-5,10-dioxabicyclo[4.4.0]dec-4-yl)oxy]-6-(hydroxy-methyl)oxane-3,4-diol) sulfate is shown in Figure 1.

Figure 1. Structure of apramycin sulfate



2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

(a) Rats

In a preliminary study, which did not comply with good laboratory practice (GLP), rats (number and sex not specified) were given a single dose of 4 mg [^{14}C]-apramycin (form not specified), either orally or subcutaneously. Radioactivity was determined in excreta and, at sacrifice 4 days after dosing, in the kidneys. The subcutaneous dose was excreted predominantly in urine (93%), with the remainder in faeces. Following an oral dose, only 0.5% was found in urine, with 99.5% in faeces, indicating very low absorption from the gastrointestinal tract. The levels of radioactivity in kidneys were 12 µg/g and 0.2 µg/g after the subcutaneous and oral doses, respectively. The primary route of excretion in rats following oral administration was in the faeces (Donoho et al., 1976).

(b) Dogs

In a 6-month non-GLP-compliant toxicity study, Beagle dogs (six animals of each sex per group) received an oral dose of 0, 25, 50 or 100 mg of apramycin activity per kilogram body weight per day in capsules (see section 2.2.2(c)). Venous blood was collected 1, 2, 3, 4, 6 and 24 hours after apramycin administration on days 1, 64, 119 and 182. Serum concentrations of apramycin peaked at 2 hours and were proportional to the dose, with means of 2.2, 5.5 and 11.0 µg/ml at doses of 25, 50 and 100 mg/kg bw per day, respectively. Essentially no apramycin was detected in plasma 24 hours after administration, and there was no evidence of accumulation or altered plasma levels following repeated administration. Urine collected for a 24-hour period after dosing on days 1, 64, 119 and 182 contained between 0.3% and 10.5% of the administered dose. The mean concentrations of apramycin in kidneys at the end of the treatment period were proportional to the dose: 45.2, 113.2 and 170 µg/g tissue at doses of 25, 50 and 100 mg/kg bw per day, respectively. Lower mean levels of apramycin were detected in kidneys of dogs

allowed a 3-month treatment-free period after 6 months of dosing: 10.2, 25.8 and 29.4 µg/g tissue at 25, 50 and 100 mg/kg bw per day, respectively (Howard et al., 1977c).

In another study that did not comply with GLP, Beagle dogs (4–5 animals of each sex per group) were given an oral dose of 0, 25, 50 or 100 mg of apramycin activity per kilogram body weight per day in capsules for 1 year (see section 2.2.2(c)). Venous blood was collected 1, 2, 3, 4 and 6 hours after apramycin administration during weeks 15, 28 and 51. Serum concentrations of apramycin peaked at 1–2 hours and were proportional to the dose; mean concentrations were between 1.12 and 3.63 µg/ml, 4.86 and 6.3 µg/ml and 5.85 and 11.9 µg/ml at doses of 25, 50 and 100 mg/kg bw per day, respectively. There was no evidence of apramycin accumulation or altered plasma levels following repeated administration. The mean concentrations of apramycin in kidneys at the end of the treatment period were proportional to the dose: 20.7, 41.9 and 82.2 µg/g tissue at 25, 50 and 100 mg/kg bw per day. The results from the studies in dogs suggested low absorption after oral dosing. The primary route of excretion in dogs was in the faeces (Howard, Johnson & Bridge, 1980).

2.1.2 Biotransformation

In the Donoho et al. (1976) study in rats described in section 2.1.1, the radioactivity was identified using thin-layer bioautography as primarily apramycin, suggesting that no metabolism had occurred.

2.2 Toxicological studies

2.2.1 Acute toxicity

(a) Single-dose toxicity

The results of non-GLP-compliant studies of the acute toxicity of different forms of apramycin are summarized in Table 1.

Following oral dosing, there were no deaths in mice, rats, rabbits or dogs. Clinical signs of toxicity were diarrhoea for 4–6 hours after dosing and cachectic appearance for 4–5 days in mice, thinness, poor grooming and diarrhoea in rats on the first day, anorexia, weight loss and hypoactivity in rabbits and mild diarrhoea and vomiting in dogs. Guinea-pigs exhibited anorexia, alopecia, distended stomach, reduced weight gain, renal injury and delayed death in 2 of 10 animals. Apramycin demonstrated low acute toxicity when administered by the oral route.

Mortality occurred within 1 hour of intravenous dosing in rodents and appeared to be due to central nervous system toxicity. The higher lethality of the sulfate forms was shown to be due to the lower pH of the dosing solutions. Mice and rats that survived high doses revealed varying degrees of renal damage.

Rats were exposed to an atmosphere containing particulate apramycin for 1 hour. There were no deaths or toxic signs at the single dose used. The median lethal concentration (LC_{50}) was greater than 211 mg apramycin activity per cubic metre (Arthur et al., 1977).

Table 1. Summary of acute toxicity (lethal dose) studies with apramycin

Apramycin form	Species, sex	Route of administration	LD_{50} (mg activity/kg bw)	References
Sulfate	Mouse, M + F	Oral	>5200	Howard & Van Duyn (1976); Worth et al. (1976c)
Sulfate	Rat, M + F	Oral	>4160	Howard & Van Duyn (1976); Worth et al. (1976c)
Sulfate	Guinea-pig, M + F	Oral	>1250	Howard & Van Duyn (1976); Worth et al. (1976c)
Sulfate	Rabbit, M + F	Oral	>832	Howard & Van Duyn (1976); Worth et al. (1976c)
Sulfate	Dog, M + F	Oral	>520	Howard & Van Duyn (1976); Worth et al. (1976c)
Base	Mouse, M + F	Intravenous	570–573	Howard & Van Duyn (1976); Worth et al. (1976b)
Base	Rat, M + F	Intravenous	1596–1640	Howard & Van Duyn (1976); Worth et al. (1976b)

F, female; LD_{50}, median lethal dose; M, male

(b) Irritation and sensitization

(i) Rabbits

Dermal application of 2000 mg apramycin sulfate (52% activity) per kilogram body weight to the intact and abraded skin of six rabbits caused slight erythema at the application site for a period of 5 days. There were no deaths or signs of systemic effects. This study was not GLP compliant (Worth et al., 1976a).

The instillation of 36 mg apramycin sulfate (52% activity) into the left eye of six rabbits caused slight conjunctival redness in two animals, which cleared within 48–96 hours. There were no deaths or signs of systemic effects. This study was not GLP compliant (Worth et al., 1976a).

(ii) Guinea-pigs

Guinea-pigs received dermal applications of 40 mg apramycin activity 3 times a week for 3 weeks for a total of 10 doses, followed by a 2-week rest period. Body weight gains were unaltered. When guinea-pigs were challenged in the fifth week, there was no evidence of skin sensitization. This study was not GLP compliant (Worth et al., 1976a).

2.2.2 Short-term studies of toxicity

(a) Mice

In two separate non-GLP-compliant studies, apramycin base was admixed in the diet and fed to B6C3F1 mice for 3 months. In the first study, doses of 0, 5000, 10 000 and 20 000 mg/kg of feed were administered to groups of 10 males and 10 females. In the second study, doses of 0 and 50 000 mg/kg of feed were given to groups of 15 males and 15 females. Dietary assays generally revealed apramycin concentrations well below the target level. Virtually no apramycin was present in the 10 000 mg/kg feed; therefore, this group was terminated early and not reported. There were no treatment-related deaths, and the sole clinical sign of toxicity was alopecia in males given 20 000 and 50 000 mg/kg feed. Body weight gains were reduced in both sexes given 50 000 mg/kg feed and in males given 20 000 mg/kg feed. Slightly decreased lymphocyte counts and increased neutrophil counts were observed at the highest dose. Blood chemistry was not meaningfully affected. Absolute and relative liver and kidney weights were lower in males and females given 50 000 mg/kg feed, but pathology was unaffected. Because the concentrations of apramycin in feed were considerably different from the expected levels, there was uncertainty as to the administered doses. Therefore, a no-observed-adverse-effect level (NOAEL) could not be determined (Howard et al., 1986).

(b) Rats

In a non-GLP-compliant study, groups of 10 male and 10 female Fischer 344 rats were fed diets containing 0, 10 000, 25 000 or 50 000 mg apramycin activity per kilogram feed for 1 month. No deaths occurred in any group. During the last week of the study, rats from the 25 000 and 50 000 mg/kg feed groups excreted dark to black faeces. Body weight gain was depressed and serum glucose and blood urea nitrogen were increased in males and females given 50 000 mg/kg feed. Also at this dose level, there were slight decreases in serum levels of creatinine in females and in alkaline phosphatase activity in males. Haematological parameters and organ weights were not examined, and only limited pathology was undertaken. No lesions were seen in the kidneys, whereas the caeca of all high-dose rats were 2–3 times normal size. Because an inadequate range of parameters was investigated, a NOAEL was not determined (Howard & Johnson, 1981).

In a non-GLP-compliant study, groups of 10 male and 10 female weanling Harlan rats were fed diets containing 0, 200, 400 or 1000 mg apramycin sulfate per kilogram feed. Another group received apramycin sulfate in the drinking-water at a concentration of 10 mg/ml. The treatments were given for a period of 3 months. The time-weighted average doses of apramycin sulfate were 0, 11, 21 and 50 mg/kg bw per day in males and 0, 13, 26 and 63 mg/kg bw per day in females treated via the diet and 1040 mg/kg bw per day in males and 1359 mg/kg bw per day in females treated via the drinking-water. All animals survived, and mild diarrhoea in the drinking-water group was the only clinical sign. There were no meaningful findings in body weight gain, ophthalmology, haematology, blood chemistry, organ weights or pathology. The no-observed-effect level (NOEL) was the highest dietary dose of 1000 mg apramycin sulfate per kilogram feed, equal to 26 and 33 mg of apramycin

activity per kilogram body weight per day in males and females, respectively (Worth et al., 1976d).

In another non-GLP-compliant 3-month study, groups of 15 male and 15 female Fischer 344 rats were fed diets containing 0, 1800, 2750, 6200 or 10 000 mg apramycin activity per kilogram feed. The time-weighted average doses were 0, 129, 198, 460 and 738 mg/kg bw per day in males and 0, 153, 228, 556 and 896 mg/kg bw per day in females. There was no mortality, and there were no effects on clinical signs, haematology, blood chemistry or organ weights. Feed consumption in all treated groups and body weight gains in females given 6200 and 10 000 mg/kg feed were slightly elevated, but not in a dose-related manner. Mild nephrosis was observed in three male rats at the highest dose. Based on kidney toxicity, the NOAEL was 6200 mg/kg feed, equal to 460 mg/kg bw per day in males (Howard & Owen, 1981).

In a non-GLP-compliant study, Wistar rats were fed diets containing 0, 1000, 2500 or 5000 mg apramycin activity per kilogram feed for up to 6 months. Groups of 4–6 rats of each sex were necropsied after 4 weeks, and groups of 10 rats of each sex were necropsied after 29–30 weeks. Drug intake during the first month was 0, 96, 236 and 461 mg/kg bw per day in males and 0, 94, 241 and 475 mg/kg bw per day in females. Over the full 6 months, the time-weighted average doses were 0, 69, 170 and 343 mg/kg bw per day in males and 0, 77, 191 and 388 mg/kg bw per day in females. There was no mortality, and there were no effects on feed consumption, body weight gain or urine analysis. Darker, softer faeces with increased moisture were found in all treated groups in a dose-related manner and were associated with a concomitant increase in water intake. Erythrocyte counts, haemoglobin and haematocrit were lower in males given 5000 mg/kg feed for 1 month, but not at the end of the study. Neutrophil counts were lower in high-dose females at the end of treatment. Serum glutamate dehydrogenase activity was increased in animals given 5000 mg/kg feed for 6 months. Absolute and relative kidney weights were lower in high-dose males, but kidney pathology was unchanged. Based on haematological and biochemical findings, the NOAEL was 2500 mg/kg feed, equal to 170 mg/kg bw per day in males and 191 mg/kg bw per day in females (Tarrant et al., 1976).

(c) Dogs

In a non-GLP-compliant study, groups of three male and three female Beagle dogs were administered 0, 5, 10 or 25 mg apramycin sulfate per kilogram body weight per day in capsules for a period of 3 months. Survival, clinical signs, body weight gain, haematology, blood chemistry, urine analysis, myeloid/erythroid ratios and pathology were unaffected. The weights of kidney, heart and testes were elevated in dogs at the highest dose when compared with concurrent controls. These apparent changes were not significantly different from historical control values. The NOEL was the highest dose of 25 mg apramycin sulfate per kilogram body weight per day, equal to 13 mg apramycin activity per kilogram body weight per day (Worth et al., 1976e).

In a non-GLP-compliant study, Beagle dogs received oral doses of 0, 25, 50 or 100 mg apramycin activity per kilogram body weight per day in capsules. Groups of four males and four females were terminated after 6 months of treatment, and

further groups of two dogs of each sex per dose were treated for 6 months and retained for a 3-month drug-free period. Body weight gain was lower in both sexes given 100 mg/kg bw per day, but no dog died during treatment. One high-dose dog had decreased appetite during the treatment phase and developed anorexia in the recovery period. As a result of weight loss and weakness, this animal was killed on day 236. During months 3–5, one dog given 50 mg/kg bw per day and four dogs given 100 mg/kg bw per day did not respond to noise stimuli, but all responded at 6 months. Soft stools occurred in nearly all treated dogs, which is likely to be a consequence of an effect on the bacterial flora rather than a toxic effect in the dog. Erythrocyte counts were consistently lower in both sexes during treatment with 50 and 100 mg/kg bw per day, but not in a dose-related manner, and statistical significance was achieved on only three of the eight occasions on which haematology was examined. Blood chemistry, urine analysis, organ weights and pathology (including electron microscopy of renal proximal convoluted tubules) were unremarkable. Based on reduced body weight gain, the NOAEL was 50 mg/kg bw per day (Howard et al., 1977c).

In a non-GLP-compliant study, Beagle dogs were given oral doses of 0, 25, 50 or 100 mg apramycin activity per kilogram body weight per day in capsules for 1 year. There were five males and four females in the control group and four dogs of each sex per dose in the treated groups. There were no effects on survival, clinical signs, ophthalmology, auditory response, body weight gain, haematology, blood chemistry, urine analysis or pathology. Adrenal weights were increased in males at the highest dose but were not associated with pathological alterations. The NOAEL was the highest dose of 100 mg/kg bw per day (Howard, Johnson & Bridge, 1980).

(d) Chickens

In a GLP-compliant study, groups of 60 male and 60 female Cobb broiler chicks received drinking-water containing 0, 500, 1500 or 2500 mg of apramycin sulfate per litre when they were 22–36 days of age. The birds were sacrificed when they were 37–44 days of age. Survival, clinical signs, body weight gain, feed consumption, haematology, blood chemistry, organ weights and pathology were unaffected (Roberts et al., 1985).

(e) Pigs

In a non-GLP-compliant study, groups of five male and five female pigs (strain not specified) were fed diets containing 0, 110, 330 or 550 mg apramycin activity per kilogram feed for 28 days. There were no effects on survival, clinical signs, body weight gain, haematology, blood chemistry, urine analysis, organ weights or pathology (Van Duyn & Zimmerman, 1978).

In another non-GLP-compliant study, groups of four male and four female weanling pigs (strain not specified) were administered drinking-water containing 0, 0.2, 0.6 or 0.9 g apramycin activity per litre for 28 days. There were no treatment-related effects on survival, body weight gain, haematology, blood chemistry, urine analysis, organ weights or pathology (Van Duyn et al., undated).

(f) Cattle

In a non-GLP-compliant study, groups of five male and five female Holstein dairy calves received 0, 20, 40, 80 or 120 mg apramycin activity per kilogram body weight per day, as apramycin sulfate, in reconstituted milk replacer for 5 days. All treated animals remained in good health throughout the study. There were no treatment-related effects on survival, body weight gain, haematology, blood chemistry, urine analysis, organ weights or pathology (Van Duyn & Johnson, 1978).

2.2.3 Long-term studies of toxicity and carcinogenicity

(a) Mice

In a GLP-compliant study, B6C3F1 mice were fed diets containing 0, 1500, 5000, 15 000 or 45 000 mg apramycin activity per kilogram feed for 2 years. Each group consisted of 60 mice of each sex. The time-weighted average doses were 0, 189, 623, 1928 and 7183 mg/kg bw per day in males and 0, 213, 668, 2043 and 7570 mg/kg bw per day in females. There were no effects on survival or clinical signs of toxicity. During the majority of the study, body weight gains were lower in males and females given 5000 mg/kg feed and above. At a dose of 45 000 mg/kg feed, haemoglobin and haematocrit were increased in both sexes, erythrocyte counts were increased in females and slightly decreased lymphocyte counts and increased neutrophil counts were observed in males. Serum alkaline phosphatase activity was increased in females given 5000–45 000 mg/kg feed. Serum glucose level was decreased and blood urea nitrogen was increased in both sexes at the highest dose. Organ weights and gross pathology were unaffected. Histopathology revealed a dose-related cytoplasmic basophilia involving the renal cortical tubular epithelium in both sexes at doses of 5000–45 000 mg/kg feed. The incidences of tumours were not increased. Based on reduced body weight gain and kidney pathology, the NOAEL was 1500 mg/kg feed, equal to 189 mg/kg bw per day in males and 213 mg/kg bw per day in females (Williams & Fisher, 1987).

(b) Rats

In a GLP-compliant study, Fischer 344 rats were maintained on diets containing 0, 2500, 5000, 10 000 or 50 000 mg apramycin activity per kilogram feed for 2 years. There were 59 males and 61 females in the control group and 50 rats of each sex per treatment group. The animals were sourced from the offspring of rats given similar diets for 3 months. The time-weighted average doses were 0, 124, 245, 488 and 2772 mg/kg bw per day for males and 0, 154, 301, 610 and 3451 mg/kg bw per day for females. Mortality, auditory response, feed consumption, haematology, blood chemistry and pathology were unaffected. Body weight gains and liver and kidney weights were reduced in both sexes at the highest dose. The incidences of tumours were not influenced by treatment. Based on reduced body weight gain and reduced organ weights, the NOAEL was 10 000 mg/kg feed, equal to 488 mg/kg bw per day in males and 610 mg/kg bw per day in females (Howard, Johnson & Owen, 1981).

Table 2. Results of tests for genotoxicity with apramycin

End-point	Test object	Concentration (form of apramycin)	Result	Reference
Reverse mutation in vitro[a]	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538	3–300 µg/plate ±S9 (apramycin sulfate)	Negative	Haworth (1979)
Reverse mutation in vitro[b]	*S. typhimurium* strains TA98, TA100, TA1535, TA1537 *Escherichia coli* strain WP2*uvrA*$^-$	0.1–1000 µg/ml ±S9 (form of apramycin not specified)	Negative	Rexroat et al. (1995)
Forward mutation in vitro[c]	Mouse lymphoma L5178Y cells	62.5–1000 µg/ml ±S9 (apramycin sulfate)	Negative	Rinzel & McMahon (1979)
DNA repair in vitro[d]	Rat hepatocytes	0.5–500 nmol/ml (apramycin sulfate)	Negative	Howard, Probst & Neal (1980)

DNA, deoxyribonucleic acid; S9, 9000 × *g* supernatant of rat liver used for metabolic activation

[a] Positive controls were 2-nitrofluorene, 1,3-propane sultone and 9-aminoacridine in the absence of S9, 2-aminoanthracene in the presence of S9.

[b] Positive controls were 2-nitrofluorene and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine in the absence of S9, 2-aminoanthracene and streptozotocin in the presence of S9.

[c] Positive controls were 2-aminofluorene and streptozotocin.

[d] Positive controls were *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine and 2-acetylaminofluorene.

2.2.4 Genotoxicity

Apramycin was evaluated for potential genotoxicity in a battery of in vitro tests. The results are summarized in Table 2. The tests included 1) in vitro assays for the induction of gene mutations in strains of *Salmonella typhimurium* and *Escherichia coli*; 2) an in vitro assay for the induction of gene mutations in L5178Y mouse lymphoma cells; and 3) an in vitro assay for the induction of deoxyribonucleic acid (DNA) repair in rat hepatocytes. The results were negative in all cases.

2.2.5 Reproductive and developmental toxicity

(a) Multigeneration studies in rats

In a GLP-compliant study, Fischer 344 rats were fed diets containing 0, 2500, 5000 or 10 000 mg apramycin activity per kilogram feed continuously over four generations. The initial groups of rats were bred on three occasions; the F_{1a} offspring were killed due to poor survival in all groups, the F_{1b} offspring were assigned to the chronic toxicity and carcinogenicity study and the F_{1c} offspring were bred to give the F_2 generation. The F_2 animals were mated twice to give the F_{3a} offspring, which were reared to weaning, and the F_{3b} pregnancy, which was terminated on gestation day 20 for examination of fetuses. Each mating group consisted of 25 males and

25 females, and the time-weighted average doses were 0, 194, 388 and 785 mg/kg bw per day. In adult rats, there were no effects on mortality, clinical signs, body weight gain, pregnancy rate or gestation length. Litter size, birth weight and sex ratio of offspring, body weight gain and survival during lactation, and gross, visceral and skeletal fetal examinations were unaffected by treatment. The NOEL was the highest dose of 10 000 mg/kg feed, equal to 785 mg/kg bw per day (Howard, Owen & Adams, 1981).

(b) Reproductive toxicity in pigs

In a non-GLP-compliant study, a single Yorkshire boar and groups of 17 sows (Yorkshire or Hampshire × Yorkshire) were given drinking-water containing 0 or 0.53 g apramycin activity per litre, as apramycin sulfate. Apramycin intake was 4 g/day for the boar and varied between 23.4 and 35.5 mg/kg bw per day for the sows. The boar was treated from 5 days prior to collection of sperm for artificial insemination. The sows were treated for 7 days at the time of insemination, on days 21–28 of gestation and on lactation days 1–7. The number of pregnancies, litter size, body weight at birth, and survival and weight gain of offspring to lactation day 14 were similar between groups (Melliere, Waitt & Paxton, undated).

(c) Developmental toxicity

(i) Rats

In a non-GLP-compliant study, groups of 25 pregnant female Wistar rats were given a gavage dose of 0, 250, 500 or 1000 mg apramycin activity per kilogram body weight per day on gestation days 6–15. The animals were killed on gestation day 20. All maternal animals survived and showed no effects on feed consumption or body weight gain. There were no intergroup differences in the numbers of implants, resorptions or dead fetuses. Fetal sex ratio, body weight and the incidences of gross, visceral and skeletal abnormalities were unaffected by treatment. There was no evidence of maternal toxicity, fetotoxicity or teratogenicity at any dose level tested. The NOEL was the highest dose tested, 1000 mg/kg bw per day (Howard et al., 1977a).

(ii) Rabbits

In a non-GLP-compliant study, groups of 15 pregnant female Dutch Belted rabbits were given a gavage dose of 0, 2, 8 or 32 mg apramycin activity per kilogram body weight per day on gestation days 6–18. The animals were killed on gestation day 28. In all treated groups, maternal feed consumption and body weight gains were markedly reduced, and the numbers of animals aborting were increased in a dose-related manner. Most of the dams aborting showed an empty gastrointestinal tract. In the remaining females, the numbers of implants and dead fetuses were similar between groups. The number of resorptions was increased at 32 mg/kg bw per day. Fetal body weight was depressed in a dose-related manner, and the incidence of bilateral 13th ribs was increased at the highest dose. The incidences of fetal malformations and external and visceral abnormalities were unaffected. The maternal toxicity was likely related to the particular sensitivity of the gut flora in

rabbits given certain antimicrobial agents (FAO/WHO, 2009); therefore, a NOEL could not be identified. The fetal toxicity was probably secondary to the severe toxicity in dams (Howard et al., 1977b).

2.2.6 Special studies

(a) Gastrointestinal irritation

In a non-GLP-compliant study, groups of four male and six female pigs (strain not specified) were fed diets containing 0 or 110 mg apramycin sulfate per kilogram for 5 weeks. There were no effects on survival, body weight gain or gross examination of the gastrointestinal tract (Van Duyn & Rathmacher, undated).

(b) Kidney toxicity

In a non-GLP-compliant study, groups of 24 female Sprague-Dawley rats were hydrated with 0.9% saline, by gavage, at 25 ml/kg bw, and urine was collected for 5 hours. Apramycin sulfate was included in the saline at a dose of 0, 0.2, 1, 2, 5, 10 or 50 mg/kg bw. The highest dose resulted in a 38% decrease in urine volume, which was associated with higher concentrations of sodium, potassium, chloride and creatinine and increased osmolality. However, the absolute amounts of each electrolyte and creatinine excreted were not significantly altered (Tust & Garrity, 1981).

(c) Ototoxicity

(i) Cats

In a non-GLP-compliant study, mongrel cats (two males and two females) received daily subcutaneous injections of 100 mg apramycin activity per kilogram body weight, approximately 1.5 hours after feed, for up to 30 days. The animals displayed anorexia and body weight loss, leading to early death or sacrifice of three cats on days 12, 16 and 25. Post-rotatory nystagmus gradually declined, from 23 to 9 seconds, in one cat, whereas the righting reflex was not affected in any cat. Pathological examination of the deceased cats revealed severe nephrosis in the kidneys (Worth et al., 1976f).

(ii) Pigs

In a non-GLP-compliant study, groups of five male and five female pigs (strain not specified) were fed diets containing 0 or 110 mg apramycin activity per kilogram for 8 weeks. Prior to commencement of dosing, the animals were conditioned to respond to an auditory stimulus to avoid an electrical shock. The response was unchanged in the treatment group (Anon, undated).

(d) Pharmacology

(i) Isolated tissues

The pharmacological activity of apramycin (10^{-8} to 10^{-5} mol/l) was investigated in isolated preparations of guinea-pig ileum, trachea and atria and rat aorta, vas

deferens and estrogen-primed uterus. The smooth and cardiac muscle tissues were suspended in an organ bath with Kreb's bicarbonate solution at 37 °C (ileum, trachea, aorta, atria, vas deferens) or Jalon's solution at room temperature (uterus) and aerated with 95% oxygen and 5% carbon dioxide. The rate of spontaneously beating guinea-pig atria was slightly increased (4%) at an apramycin concentration of 10^{-5} mol/l (5.4 µg apramycin activity per millilitre), but agonist activity was not observed in any other tissue. At a concentration of 10^{-5} mol/l, apramycin slightly enhanced the response to carbamylcholine (cholinergic agonist) in guinea-pig ileum and slightly inhibited the response to phenylephrine (sympathomimetic α_1 agonist) in rat aorta. The responses to histamine in the guinea-pig ileum, norepinephrine in rat vas deferens, oxytocin in rat uterus and isoproterenol in guinea-pig trachea were not changed, indicating that apramycin did not block the muscarinic, histamine H_1 or β-adrenergic receptors. These experiments were not GLP compliant (Ruffolo & Beckhelm, 1981).

(ii) Rats

In a non-GLP-compliant study, anaesthetized rats were given single doses of apramycin at 25 mg/kg bw intravenously or at 250 and 400 mg/kg bw by gavage. The twitch response of the anterior tibialis muscle, resulting from stimulation of the sciatic nerve, was unaffected at each dose, indicating no activity at the neuromuscular junction. The positive control was (+)-tubocurarine (Ruffolo, 1980).

(iii) Dogs

In a GLP-compliant study, anaesthetized dogs received intravenous infusions of apramycin at cumulative doses of 0, 4, 8, 12, 16 and 20 mg apramycin activity per kilogram body weight. Mean arterial blood pressure was increased at 8–20 mg/kg bw, but not at 4 mg/kg bw. Heart rate declined in saline controls but was maintained at doses of 8–20 mg/kg bw. These changes were abolished when animals were pretreated with either phenoxybenzamine (α-receptor antagonist) or propranolol (β-receptor antagonist), suggesting a minor sympathomimetic action for apramycin. There were no alterations in electrocardiogram, cardiac output, stroke work, vascular resistance, respiratory parameters or blood gases at any dose (Holland et al., 1981).

(e) Microbiological effects

A JECFA decision-tree approach that was adopted by the sixty-sixth meeting of the Committee (Annex 1, reference *181*) and that complies with International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) Guideline 36 (GL36) was used by the Committee to determine the need to establish a microbiological ADI for apramycin (VICH, 2004). The decision-tree approach initially seeks to determine if there may be microbiologically active apramycin residues entering the human colon. If the answer is "no" to any of the first three steps, then no microbiological ADI is necessary. However, should such residues be present, then two end-points of public health concern are to be considered: 1) disruption of the colonization barrier and 2) increase of the population(s) of resistant bacteria. At Step 4 of the

decision-tree process, it is possible to provide scientific justification to eliminate testing (i.e. the need for a microbiological ADI) for either one or both end-points. At Step 5, a microbiological ADI would be determined. Should a microbiological ADI not be necessary, then the toxicological or pharmacological ADI would be used. The Committee evaluated a GLP-compliant study of minimum inhibitory concentration (MIC) susceptibility testing and studies on the biological activity of apramycin residue and used the decision-tree to answer the following questions in the assessment of apramycin.

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

Yes. Apramycin is microbiologically active against some bacterial genera and species representative of the human intestinal microflora. In radiolabel studies, no apramycin metabolites were identified.

The MIC of apramycin was determined against 100 bacterial strains, comprising 10 isolates from each of 10 groups of genera representing the normal intestinal microbiota: *Bifidobacterium*, *Eubacterium*, *Clostridium*, *Bacteroides fragilis*, other *Bacteroides* species ("non-*fragilis* group"), *Fusobacterium*, *Peptostreptococcus*, *Lactobacillus*, *Enterococcus* and *Escherichia coli* (Pridmore & Cheetham, 2011). All strains were sourced from faecal samples of healthy, unmedicated humans. Each volunteer experienced no symptoms of diarrhoea within the 4-week period preceding sample collection and no antimicrobial therapy during the 3-month period prior to sample collection. The test system was standardized agar dilution MIC methodology using quality control strains as described in the Clinical and Laboratory Standards Institute guidelines (CLSI, 2004).

Apramycin activity against each bacterial group is summarized in Table 3. MIC_{50}, MIC_{90}, geometric mean MIC and MIC range were calculated for each bacterial group. Apramycin activity was clearly shown against *Escherichia coli* (MIC_{50} = 4 µg/ml). Apramycin exerted no measurable antibacterial activity (MIC values > 128 µg/ml) against *Bifidobacterium*, *Clostridium*, *Bacteroides fragilis* and "non-*fragilis*" *Bacteroides* strains. Apramycin exerted relatively poor activity against *Lactobacillus* (MIC_{50} = 64 µg/ml), *Enterococcus* (MIC_{50} = 32 µg/ml), *Fusobacterium* (MIC_{50} = 16 µg/ml), *Eubacterium* (MIC_{50} = 16 µg/ml) and *Peptostreptococcus* (MIC_{50} = 16 µg/ml).

Step 2: Do residues enter the human colon?

Yes. A number of residue and bioavailability studies using [^{14}C]apramycin and analytical (high-performance liquid chromatography) and microbiological assay methods were conducted to detect apramycin in pigs, cattle and poultry. Muscle, kidney, fat and skin contain little or no apramycin-derived residues, regardless of the period between withdrawal of medication and slaughter, for the various routes of administration. However, apramycin residues were present at low levels in the liver. Therefore, apramycin residues may be present at low levels in meat products consumed by humans. Apramycin is poorly absorbed from the gastrointestinal tract; therefore, residues could enter the colon of a person ingesting edible tissues from treated animals.

Table 3. Susceptibility of representative human intestinal bacteria to apramycin

Bacterial group	Summary of apramycin MIC parameters (µg/ml)			
	MIC range	MIC_{50}	MIC_{90}	Geometric mean MIC
Bifidobacterium spp.	All >128	>128	>128	>128
Eubacterium and related species	4 to >128	16	>128	26
Clostridium spp.	All >128	>128	>128	>128
Bacteroides fragilis group	All >128	>128	>128	>128
Bacteroides (non-*fragilis* group)	128 to >128	>128	>128	>128
Fusobacterium spp.	2 to >128	16	>128	34
Peptostreptococcus spp.	2 to >128	16	128	13
Lactobacillus spp.	32 to 128	64	64	60
Enterococcus spp.	32 to 128	32	64	37
Escherichia coli	4 to 8	4	4	4.3
All strains (*n* = 100)	2 to >128	128	>128	44

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

Yes. As the studies submitted for evaluation did not contain measurements of the amounts of drug residue in the intestinal tract, the Committee used the pharmacokinetic and bioavailability studies to determine the fraction of oral dose available to the human intestinal microflora. Pharmacokinetic and bioavailability studies indicated that apramycin is poorly absorbed after oral administration. A considerable amount of the administered apramycin was detected as unmetabolized parent compound in faecal samples. No metabolites were identified in any of the food animals dosed with apramycin. Therefore, apramycin residues entering the human colon will remain microbiologically active.

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

Yes. The Committee noted that there is scientific justification to eliminate resistance development as an end-point of concern. However, there is the potential for an adverse effect on disruption of the colonization barrier of the human gastrointestinal tract, as apramycin residues do occur in chicken, pigs, sheep, calves and rabbits, and, if edible tissues are ingested, they could have an impact on the intestinal microbiota. Furthermore, diarrhoea occurred in toxicity studies in mice, rats, guinea-pigs, rabbits and dogs after oral administration of apramycin, suggesting adverse effects of apramycin on the intestinal microbiota.

In terms of antimicrobial resistance, results from microbiological studies indicate that resistance was shown to occur in various bacteria isolated from animals treated with apramycin (Mortensen et al., 1996). Although apramycin is solely used in veterinary medicine, cross-resistance to other aminoglycosides has been documented (Pohl et al., 1993; Johnson et al., 1994; Yates et al., 2004; Jensen et al., 2006). Apramycin is recommended for the treatment of intestinal bacterial infections in animals, including colibacillosis. Apramycin-resistant *E. coli* have been isolated from farm animals and farm workers (Zhang, Ding & Fan, 2009).

Therefore, apramycin could modify the intestinal microbiota by creating a selective pressure favouring the development of apramycin-resistant bacteria. However, as the majority of apramycin residue levels detected in target tissues were below the lowest MIC_{50} value of any of the representative human intestinal microbiota listed in Table 3 and the clinical breakpoint for *E. coli* is 32 µg/ml, it is unlikely that the development of resistance to apramycin residues and cross-resistance to commonly used aminoglycosides in veterinary and human medicine would occur.

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

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

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

The equation terms are derived as described below.

MIC_{calc}: In accordance with Appendix C of VICH GL36, calculation of the estimated no-observed-adverse-effect concentration (NOAEC) (MIC_{calc}) for colonization barrier disruption uses MIC values from the lower 90% confidence limit of the mean MIC_{50} for the most relevant and sensitive human colonic bacterial genera. The strains needed to determine the MIC_{calc} were chosen according to these guidelines, which state that an intrinsically resistant bacterial genus should not be included. Based on the genera with a MIC_{50} of 32 µg/ml or less (i.e. *Escherichia coli*, *Enterococcus*, *Fusobacterium*, *Peptostreptococcus* and *Eubacterium* species), the MIC_{calc} is 8.3 µg/ml.

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

Fraction of oral dose available to microorganisms: It is recommended that the fraction of an oral dose available for colonic microorganisms be based on in vivo measurements for the drug administered orally. Alternatively, if sufficient data are available, the fraction of the dose available for colonic microorganisms can be calculated as 1 minus the fraction (of an oral dose) excreted in urine. Human data are encouraged, but, in their absence, non-ruminant animal data are recommended. In the absence of data to the contrary, it should be assumed that metabolites have antimicrobial activity equal to that of the parent compound. The fraction may be

lowered if the applicant provides quantitative in vitro or in vivo data to show that the drug is inactivated during transit through the intestine. Apramycin is poorly absorbed and is excreted in faeces primarily in unchanged form; therefore, the value is 1.

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

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

$$\text{Upper bound of ADI} = \frac{8.3\ \mu g/ml \times 220\ g}{1 \times 60\ kg\ bw}$$

$$= 30.4\ \mu g/kg\ bw$$

Therefore, a microbiological ADI of 0–30 µg/kg bw (rounded to one significant figure) could be derived from in vitro MIC susceptibility testing.

2.3 Observations in humans

Apramycin has not been used in the treatment of humans. No data could be identified relating to effects in humans.

3. COMMENTS

In addition to a submission from the sponsor, a literature search was conducted, which identified only one further study report. The Committee considered the results of studies on pharmacokinetics, acute, short-term and long-term toxicity, genotoxicity, reproductive and developmental toxicity, pharmacology, ototoxicity, kidney toxicity and microbiological safety. A number of critical studies, namely the 2-year studies in mice and rats, the multigeneration reproductive toxicity study in rats, two of the four genotoxicity studies and the microbiology studies, were carried out according to appropriate standards. The majority of the other studies were performed prior to the establishment of standards for study protocol and conduct.

3.1 Biochemical data

In a preliminary study in rats, an oral dose of apramycin was poorly absorbed from the gastrointestinal tract, and approximately 99.5% was excreted unchanged in the faeces. Primary data were not supplied for this study.

In 6-month and 12-month toxicity studies in dogs given daily oral doses, absorption was low and was proportional to the dose. Essentially no apramycin was detected in serum at 24 hours, and there was no evidence of accumulation following repeated administration. Between 0.3% and 10.5% of the administered dose was excreted in urine, which represents the absorbed fraction. The primary route of excretion was in the faeces.

3.2 Toxicological data

Apramycin sulfate exhibited low acute toxicity by the oral route, which was probably a reflection of low absorption. The LD_{50} values (as apramycin activity)

were greater than 4000 mg/kg bw in mice and rats, greater than 1250 mg/kg bw in guinea-pigs, greater than 832 mg/kg bw in rabbits and greater than 520 mg/kg bw in dogs. High doses in mice, rats, guinea-pigs, rabbits and dogs resulted in gastrointestinal effects that were presumably related to antimicrobial activity on the intestinal microflora. Renal injury was also observed in guinea-pigs. In mice and rats, single intravenous doses of apramycin base were more toxic than oral doses, and animals showed signs of central nervous system toxicity and renal damage.

In a 3-month study in which mice were fed diets containing 0, 5000, 10 000, 20 000 or 50 000 mg of apramycin activity per kilogram, dietary assays generally revealed apramycin concentrations well below the target level. Virtually no apramycin was present in the feed containing a nominal concentration of 10 000 mg/kg. Alopecia was observed in males given 20 000 and 50 000 mg/kg feed. Body weight gain was reduced in both sexes given 50 000 mg/kg feed and in males given 20 000 mg/kg feed. Slightly decreased lymphocyte counts and increased neutrophil counts were observed at the highest dose. Absolute and relative liver and kidney weights were lower in males and females given 50 000 mg/kg feed. Because of the uncertainty in the administered doses, a NOAEL could not be determined.

In a 1-month study, rats were fed diets containing 0, 10 000, 25 000 or 50 000 mg apramycin activity per kilogram. During the last week of the study, rats from the 25 000 and 50 000 mg/kg feed groups excreted dark to black faeces. Body weight gain was depressed and blood glucose and blood urea nitrogen were increased in males and females given 50 000 mg/kg feed. Also at this dose level, there were slight decreases in serum levels of creatinine in females and in alkaline phosphatase activity in males. Haematological parameters were not examined, and only limited pathology was undertaken. The caeca of all high-dose rats were 2–3 times normal size. Because only a limited range of parameters was investigated, a NOAEL was not determined.

In a 3-month study, rats were fed diets containing 0, 200, 400 or 1000 mg apramycin sulfate per kilogram. Another group received apramycin sulfate in the drinking-water at a concentration of 10 mg/ml. Mild diarrhoea in the drinking-water group was the only finding. The NOEL was the highest dietary dose of 1000 mg apramycin sulfate per kilogram feed, equal to 26 mg of apramycin activity per kilogram body weight per day.

In another 3-month study, rats were fed diets containing 0, 1800, 2750, 6200 or 10 000 mg apramycin activity per kilogram. Feed consumption in all treated groups and body weight gains in females given 6200 and 10 000 mg/kg feed were slightly elevated, but not in a dose-related manner. Mild nephrosis was observed in some male rats at the highest dose. Based on kidney toxicity, the NOAEL was 6200 mg/kg feed, equal to 460 mg of apramycin activity per kilogram body weight per day.

In a 6-month study, rats were fed diets containing 0, 1000, 2500 or 5000 mg apramycin activity per kilogram. Darker, softer faeces with increased moisture were found in all treated groups in a dose-related manner and were associated with a concomitant increase in water intake. Erythrocyte counts, haemoglobin and haematocrit were lower in males given 5000 mg/kg feed for 1 month, but not at

the end of the study. Serum glutamate dehydrogenase activity was increased in animals given 5000 mg/kg feed for 6 months. Absolute and relative kidney weights were lower in high-dose males. Based on haematological and biochemical findings, the NOAEL was 2500 mg/kg feed, equal to 170 mg apramycin activity per kilogram body weight per day.

In a 3-month study, dogs were administered 0, 5, 10 or 25 mg apramycin sulfate per kilogram body weight per day in capsules. There were no treatment-related effects. The NOEL was the highest dose of 25 mg apramycin sulfate per kilogram body weight per day, equal to 13 mg apramycin activity per kilogram body weight per day.

In a 6-month study, dogs received oral doses of 0, 25, 50 or 100 mg apramycin activity per kilogram body weight per day in capsules. Body weight gain was lower in both sexes given 100 mg/kg bw per day. One dog out of eight at the high dose had decreased appetite and developed anorexia; as a result of weight loss and weakness, this animal was killed. During months 3–5, one dog of eight given 50 mg/kg bw per day and four dogs of eight given 100 mg/kg bw per day did not respond to noise stimuli, but all responded at 6 months. Soft stools occurred in all treated dogs, which is likely to be a consequence of an effect on the bacterial flora rather than a toxic effect in the dog. Erythrocyte counts were consistently lower in both sexes during treatment with 50 and 100 mg/kg bw per day, but not always in a dose-related manner and rarely statistically significantly; consequently, they were considered unrelated to treatment. Based on reduced body weight gain, the NOAEL was 50 mg apramycin activity per kilogram body weight per day.

In a 1-year study, dogs were given oral doses of 0, 25, 50 or 100 mg apramycin activity per kilogram body weight per day in capsules. Adrenal weights were increased in males at the highest dose, but were not associated with pathological alterations. The NOAEL was the highest dose of 100 mg apramycin activity per kilogram body weight per day.

In a long-term study in which mice were fed diets containing 0, 1500, 5000, 15 000 or 45 000 mg apramycin activity per kilogram for 2 years, body weight gains were lower in males and females given 5000 mg/kg feed and above. At a dose of 45 000 mg/kg feed, haemoglobin and haematocrit were increased in both sexes, erythrocyte counts were increased in females and slightly decreased lymphocyte counts and increased neutrophil counts were observed in males. Serum alkaline phosphatase activity was increased in females given 5000 mg/kg feed and higher. Serum glucose level was decreased and blood urea nitrogen was increased in both sexes at the highest dose. Cytoplasmic basophilia involving the renal cortical tubular epithelium was observed in both sexes at doses of 5000 mg/kg feed and higher. The incidences of tumours were not increased. Based on reduced body weight gain and kidney pathology, the NOAEL was 1500 mg/kg feed, equal to 189 mg apramycin activity per kilogram body weight per day. There was no evidence for carcinogenicity in mice.

In a study in which rats were given diets containing 0, 2500, 5000, 10 000 or 50 000 mg apramycin activity per kilogram for 2 years, body weight gains and liver and kidney weights were reduced in both sexes at the highest dose. The incidences

of tumours were not influenced by treatment. Based on reduced body weight gain and reduced organ weights, the NOAEL was 10 000 mg/kg feed, equal to 488 mg apramycin activity per kilogram body weight per day. There was no evidence for carcinogenicity in rats.

Apramycin was evaluated for potential genotoxicity in two in vitro assays for the induction of gene mutations in strains of *Salmonella typhimurium* and a strain of *Escherichia coli*, an in vitro assay for the induction of gene mutations in L5178Y mouse lymphoma cells and an in vitro assay for the induction of DNA repair in primary cultures of rat hepatocytes. The results were negative in all cases.

Because the carcinogenicity and genotoxicity studies were negative, the Committee concluded that apramycin is unlikely to pose a carcinogenic risk to humans.

In a multigeneration reproductive toxicity study, rats were fed diets containing 0, 2500, 5000 or 10 000 mg apramycin activity per kilogram continuously over four generations. No adverse effects on fertility and reproductive indices or on growth and survival of offspring were found. The NOEL was the highest dose of 10 000 mg/kg feed, equal to 785 mg apramycin activity per kilogram body weight per day.

In a developmental toxicity study, pregnant female rats were given a gavage dose of 0, 250, 500 or 1000 mg apramycin activity per kilogram body weight per day on gestation days 6–15. There were no effects on maternal toxicity, fetal growth and development or the incidences of fetal abnormalities. The NOEL was the highest dose of 1000 mg apramycin activity per kilogram body weight per day.

In another developmental toxicity study, pregnant female rabbits were given a gavage dose of 0, 2, 8 or 32 mg apramycin activity per kilogram body weight per day on gestation days 6–18. Maternal feed consumption and body weight gains were markedly reduced, and the numbers of animals aborting were increased, in a dose-related manner. Most of the dams aborting showed an empty gastrointestinal tract. The number of resorptions was increased at 32 mg/kg bw per day. Fetal body weight was depressed in a dose-related manner, and the incidence of bilateral 13th ribs was increased at the highest dose. The incidences of fetal malformations and external and visceral abnormalities were unaffected. The maternal toxicity was likely related to the particular sensitivity of the gut flora in rabbits given certain antimicrobial agents (FAO/WHO, 2009), and therefore it was not possible to identify a NOEL. The fetal toxicity was probably secondary to the severe toxicity in the dams.

In a special study to assess renal toxicity in rats, urine volume was decreased (38%) by a single oral apramycin sulfate dose of 50 mg/kg bw. However, the absolute amounts of urinary electrolytes and creatinine excreted were unchanged. Other special studies indicated that ototoxicity and intrinsic pharmacological activity would not be expected.

No data could be identified relating to effects in humans.

The most relevant study for determining a toxicological ADI was the 6-month study in dogs. The NOAEL was 50 mg/kg bw per day, based on reduced body

weight gain. A safety factor of 100 was considered appropriate. Therefore, an ADI of 0–0.5 mg/kg bw could be established on the basis of the toxicological data.

3.3 Microbiological data

A JECFA decision-tree approach that was adopted by the sixty-sixth meeting of the Committee (Annex 1, reference *181*) and that complies with VICH GL36 was used by the Committee to determine the need for, and to establish, if necessary, a microbiological ADI for apramycin. Studies of microbiological activity against bacterial strains representative of the human colonic flora were evaluated. Apramycin was active against *Escherichia coli* (MIC_{50} = 4 µg/ml). Apramycin exerted no measurable antibacterial activity (MIC values > 128 µg/ml) against *Bifidobacterium*, *Clostridium*, *Bacteroides fragilis* and "non-*fragilis*" *Bacteroides* strains. Apramycin exerted relatively poor activity against *Lactobacillus* (MIC_{50} = 64 µg/ml), *Enterococcus* (MIC_{50} = 32 µg/ml), *Fusobacterium* (MIC_{50} = 16 µg/ml), *Eubacterium* (MIC_{50} = 16 µg/ml) and *Peptostreptococcus* (MIC_{50} = 16 µg/ml).

Apramycin residues may be present at low levels in meat products consumed by humans; therefore, apramycin-related residues could enter the colon of a person ingesting edible tissues from treated animals. As the data submitted for evaluation did not contain measurements of the amount of drug residue in the intestinal tract, the Committee used the pharmacokinetic studies to determine the fraction of the oral dose available to the human intestinal microbiota. Apramycin was poorly absorbed after oral administration in animals, and a considerable amount of the administered apramycin was detected as unmetabolized parent compound in faecal samples. Therefore, apramycin residues entering the human colon will remain microbiologically active.

There is potential for disruption of the colonization barrier of the human gastrointestinal tract. Diarrhoea occurred in some toxicity studies in animals after oral administration of apramycin, suggesting adverse effects of apramycin on the intestinal microbiota. Because the majority of apramycin residue levels detected in target tissue were below the lowest MIC_{50} of any of the representative human intestinal microbiota tested and the clinical breakpoint for *E. coli* is 32 µg/ml, it is unlikely that the development of resistance to apramycin residues would occur.

The formula for calculating the microbiological ADI is as follows:

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

The equation terms are derived as described below.

MIC_{calc}: In accordance with Appendix C of VICH GL36, calculation of the estimated NOAEC (MIC_{calc}) for colonization barrier disruption uses MIC values from the lower 90% confidence limit of the mean MIC_{50} for the most relevant and sensitive human colonic bacterial genera. The strains needed to determine the MIC_{calc} were chosen according to these guidelines, which state that an intrinsically resistant bacterial genus should not be included. Based on the genera with a MIC_{50} of 32 µg/ml or

less (i.e. *Escherichia coli*, *Enterococcus*, *Fusobacterium*, *Peptostreptococcus* and *Eubacterium* species), the MIC_{calc} is 8.3 µg/ml.

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

Fraction of oral dose available to microorganisms: It is recommended that the fraction of an oral dose available for colonic microorganisms be based on in vivo measurements for the drug administered orally. Alternatively, if sufficient data are available, the fraction of the dose available for colonic microorganisms can be calculated as 1 minus the fraction (of an oral dose) excreted in urine. Human data are encouraged, but, in their absence, non-ruminant animal data are recommended. In the absence of data to the contrary, it should be assumed that metabolites have antimicrobial activity equal to that of the parent compound. The fraction may be lowered if the applicant provides quantitative in vitro or in vivo data to show that the drug is inactivated during transit through the intestine. Apramycin is poorly absorbed and is excreted in faeces primarily in unchanged form; therefore, the value is 1.

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

Therefore, the upper bound of the microbiological ADI for apramycin is calculated as indicated below:

$$\text{Upper bound of ADI} = \frac{8.3\ \mu g/ml \times 220\ g}{1 \times 60\ kg\ bw}$$

$$= 30.4\ \mu g/kg\ bw$$

Therefore, a microbiological ADI of 0–30 µg/kg bw (rounded to one significant figure) could be derived from in vitro MIC susceptibility testing.

4. EVALUATION

The Committee considered that microbiological effects were more appropriate than toxicological effects for the establishment of an ADI for apramycin. Therefore, the Committee established an ADI of 0–30 µg/kg bw on the basis of the data for disruption of the colonization barrier.

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