EVALUATION OF CERTAIN VETERINARY DRUG RESIDUES IN FOOD

Fiftieth report of the Joint FAO/WHO Expert Committee on Food Additives

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CORRIGENDUM

Page 76, Table 6, footnote a:

Delete The estimated total daily production in adults is 107 ng.

Insert The estimated total daily production in adults is 10^7 ng.
Joint FAO/WHO Expert Committee on Food Additives
Rome, 17–26 February 1996

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Monographs containing summaries of relevant data and toxicological evaluations are available from WHO under the title:


Residues monographs are issued separately by FAO under the title:

*Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/11, in press.

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**INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY**

The preparatory work for toxicological evaluations of food additives and contaminants by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is actively supported by certain of the Member States that contribute to the work of the International Programme on Chemical Safety (IPCS).

The International Programme on Chemical Safety (IPCS) is a joint venture of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. One of the main objectives of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment.
1. **Introduction**

A meeting of the Joint FAO/WHO Expert Committee on Food Additives was held at FAO Headquarters, Rome, from 17 to 26 February 1998. The meeting was opened by Dr D. Harcharik, Deputy Director-General, FAO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization.

Dr Harcharik drew the attention of the Committee to the review by the World Trade Organization of a trade dispute between the European Union and the United States of America concerning the use of growth-promoting hormones in meat production. A World Trade Organization Panel had to decide whether a ban on the import of beef from cattle treated with growth-promoting hormones was proper, based on the Agreement on the Application of Sanitary and Phytosanitary Measures. One of the articles in the Agreement states that these measures “are based on an assessment, as appropriate to the circumstances, of the risks to human, animal or plant life or health, taking into account risk assessment techniques developed by the relevant international organizations” (1). The hormone substances involved in the dispute had been evaluated by the Committee 10 years ago. Dr Harcharik noted that this dispute clearly emphasizes the importance and possible impact of the work of the Committee on international trade.

Ten previous meetings of the Committee had been held to consider veterinary drug residues in food (Annex 1, references 80, 85, 91, 97, 104, 110, 113, 119, 125 and 128) in response to the recommendations of the Joint FAO/WHO Expert Consultation held in 1984 (2). The present meeting was convened in response to the recommendation made at the forty-eighth meeting of the Committee that meetings on this subject should be held annually (Annex 1, reference 128). The Committee’s purpose was to provide guidance to FAO and WHO Member States and to the Codex Alimentarius Commission on public health issues pertaining to residues of veterinary drugs in foods of animal origin. The specific tasks before the Committee were:

- to elaborate further principles for evaluating the safety of residues of veterinary drugs in food and for establishing Acceptable Daily Intakes (ADIs) and Maximum Residue Limits (MRLs) for such

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1 As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives held in 1955 (FAO Nutrition Meeting Report Series, No. 11, 1956; WHO Technical Report Series, No. 107, 1956), there have been 48 previous meetings of the Joint FAO/WHO Expert Committee on Food Additives (Annex 1).
residues when the drugs under consideration are administered to food-producing animals in accordance with good practice in the use of veterinary drugs (see section 2); and

— to evaluate the safety of residues of certain veterinary drugs (see section 3 and Annex 2).

2. General considerations

2.1 Modification of the agenda

Cyhalothrin and olaquindox were removed from the agenda following requests by the sponsors for postponement of the evaluations. Porcine somatotropin was withdrawn due to the late arrival of data. Dexamethasone was added to the agenda because the analytical method for monitoring purposes, which had been requested at the forty-second meeting of the Committee (Annex 1, reference 110), was submitted for review. Chlortetracycline, oxytetracycline and tetracycline were included in the agenda at the request of the Codex Committee on Residues of Veterinary Drugs in Foods at its Tenth Session (3).

Abamectin was evaluated at the 1997 Joint FAO/WHO Meeting on Pesticide Residues, which recommended that the Committee “suggest an appropriate maximum residue level in cattle meat and consider accepting the broader definition of the residue to accommodate the residues which occur as a result of aquacultural as well as veterinary uses” (4). The Committee postponed discussion of this issue to the next meeting at which veterinary drugs are considered, pending consideration of the general items outlined in section 2.3.

2.2 Neurotoxicity of avermectins and milbemycins

At its present meeting, the Committee considered eprinomectin, an anthelmintic belonging to the avermectin class of compounds.

Two other anthelmintics of the avermectin and milbemycin classes of compounds (doramectin and moxidectin) were toxicologically evaluated at the forty-fifth meeting of the Committee (Annex 1, reference 119). At that meeting, the Committee noted that a subpopulation of CF-1 mice was highly sensitive to the neurotoxic effects of avermectins. This was due to a deficiency of P-glycoprotein, a component of the plasma membrane which controls the rate of passage of xenobiotics across membranes. The Committee also noted that some collie dogs and Murray Grey cattle are highly sensitive to the neurotoxic effects of certain avermectins. Despite the widespread
use of this class of compound in humans without apparent adverse effects, the Committee could not exclude the possibility that some sensitive human populations may exist, and that the CF-1 mouse might serve as a model for these groups. At its forty-fifth meeting, the Committee therefore concluded that in the absence of studies in CF-1 mice, the safety factor normally applied in calculating the ADI should be doubled to allow for the possible existence of some sensitive human populations (Annex 1, reference 119, section 2.2).

Since the forty-fifth meeting, additional data have become available on the expression of P-glycoprotein in the brain (and jejunum) of humans (fetuses and adults), rats (fetuses, pups, young adults and adults) and rhesus monkeys (fetuses and juveniles), and in the placenta of humans and rhesus monkeys. These data were reviewed by the 1997 Joint FAO/WHO Meeting on Pesticide Residues when abamectin was re-evaluated (4). The Joint Meeting concluded that the increased sensitivity of homozygous CF-1 mice and postnatal rats to avermectins was due to reduced expression of P-glycoprotein in these species and that the use of the results of studies with CF-1 mice was not appropriate in establishing the ADI for avermectins.

On the basis of this information, the Committee, at its present meeting, concluded that the use of an additional safety factor of 2 is no longer necessary for avermectins and milbemycins not tested in CF-1 mice.

2.3 Evaluation policy of the Committee in the establishment of MRLs for veterinary drugs in food

To promote consistency and transparency in its procedure for setting MRLs the Committee decided that it would, at future meetings, further consider and report, as appropriate, on the following general policy issues:

— principles for recommending MRLs;
— selection of units in which to express MRLs;
— establishment of MRLs using statistical approaches;
— establishment of MRLs for edible tissues and products of minor species when MRLs have been established for one or more major species;
— establishment of ADIs and MRLs for substances intended solely for use in minor animal species;
— establishment of MRLs for drugs used in aquaculture;
— selection of target tissues for which MRLs are to be established;
— establishment of MRLs for lipid-soluble residues in milk and muscle tissue based on fat content;
— establishment of MRLs for substances used both as pesticides and as veterinary drugs;
— validation of analytical methods used in residue-depletion studies and for monitoring compliance with established MRLs; and
— development of a decision-tree approach for evaluating antimicrobial effects on the human intestinal microflora.

3. Comments on residues of specific veterinary drugs

The Committee considered for the first time one anthelminthic agent, two antimicrobial agents and two antiprotozoal agents. It reconsidered four anthelminthic agents, five antimicrobial agents, one antiprotozoal agent, one glucocorticosteroid, one production aid and one tranquillizing agent. The recommendations made with regard to these substances and details of further studies and other information required are summarized in Annex 2.

Toxicological monographs or monograph addenda were prepared on all of the compounds considered in this section on which toxicological and/or microbiological evaluations were performed except for procaine benzylpenicillin. Residues monographs were prepared on all substances reviewed in this section except diclazaril and azaperone.

3.1 Anthelminthic agents

3.1.1 Eprinomectin

Eprinomectin had not been previously evaluated by the Committee.

Eprinomectin, a semisynthetic analogue of the avermectin class of compounds, is a mixture of two closely related substances differing from each other by only one methylene group in a side-chain substituent. The mixture contains not less than 90% of eprinomectin B₁₄ and not more than 10% of eprinomectin B₁₄. Eprinomectin is applied topically as a pour-on formulation. It is used as an endo- or ectoparasiticide on both beef and lactating dairy cattle. Eprinomectin is more hydrophilic than either abamectin or ivermectin while retaining the antiparasitic properties of the avermectins.

Toxicological data

The Committee considered the results of studies on the pharmacokinetics, metabolism, acute and short-term toxicity, genotoxicity and reproductive toxicity of eprinomectin. All pivotal studies were carried out according to appropriate standards for study protocol and conduct.
When radiolabelled eprinomectin was administered orally to rats, the radioactivity was found mainly in the gastrointestinal tract, followed by liver, fat and kidney, while lower levels were found in muscle and blood. Elimination occurred almost exclusively in the faeces. For up to 24 hours after drug administration, the major residue in tissues, plasma and faeces was unchanged eprinomectin. After 2–5 days the major residue was N-deacetylated B1a. The primary route of metabolism of eprinomectin in rats is N-deacetylation, and minor routes are hydroxylation and hydroxymethylation. Metabolism is more extensive in female than in male rats.

In a study in cattle, radiolabelled eprinomectin was found to be absorbed slowly after topical administration. The absorbed radioactivity was taken up mainly by the liver, and to a lesser extent by the kidney, fat and muscle. The levels of radioactivity in these tissues declined with a half-life of 7.8–8.6 days, except in muscle beneath the application site in which the half-life was 36 days. Elimination was mostly in the faeces. At all slaughter times, the major residue in tissues, plasma and faeces was unchanged eprinomectin (accounting for >85% of the total residues), with B1a representing more than 80%. The level of B1a declined in parallel with the total residues in all tissues at all slaughter times, with a half-life of 7.5–9.6 days in liver, kidney, fat and muscle, and 29 days in muscle beneath the application site. The profile of metabolites in cattle was qualitatively similar to that in rats.

After oral administration of eprinomectin, the approximate LD50 values were 70mg/kg of body weight for mice and 55mg/kg of body weight for rats. Eprinomectin was moderately hazardous following acute oral exposure.

In a 90-day toxicity study, rats received eprinomectin in the diet at nominal doses of 0, 1, 5 or 30mg/kg of body weight per day. The highest dose was lowered to 20mg/kg of body weight per day during the study because of severe toxicity at 30mg/kg of body weight per day. Male and female rats in the highest-dose group showed tremors and very slight degeneration of the sciatic nerves, as well as decreased body-weight gain and changes in organ weights. In females in the highest-dose group, arrest of normal maturation of the ovarian follicles, endometrial squamous metaplasia, and decreased remodelling of the femora (primary spongiosa), indicative of estrogenic/progestational imbalance, were also observed. The NOEL was 5mg/kg of body weight per day, on the basis of effects on the central nervous system and other effects.

In a 90-day toxicity study, dogs received eprinomectin by gavage at doses of 0, 0.4, 0.8 or 2.4mg/kg of body weight per day. The highest
dose of 2.4 mg/kg of body weight per day produced mydriasis, emesis, ataxia, salivation, lateral recumbency, loss of body-weight or death. After reduction of the dose to 1.6 mg/kg of body weight per day, decreased food consumption and decreased body-weight gain were observed in males and females. Females in the highest-dose group had very slight degeneration of sciatic nerve axons. The NOEL was 0.8 mg/kg of body weight per day on the basis of degeneration of sciatic nerve axons and body-weight loss.

In a 1-year toxicity study, dogs received eprinomectin by gavage at doses of 0, 0.5, 1 or 2 mg/kg of body weight per day. Treatment-related effects were observed only at the highest dose; these included mydriasis and very slight focal neuronal degeneration in the pons area and cerebellar nuclei of the brain. On the basis of these effects, the NOEL was 1 mg/kg of body weight per day.

Eprinomectin has been tested in vitro for its ability to induce mutations in Salmonella typhimurium and Escherichia coli, gene mutations in Chinese hamster lung cells, chromosomal aberrations in Chinese hamster ovary cells, and breaks in single-stranded DNA in primary rat hepatocytes. It has also been tested in vivo for its ability to induce micronuclei in the bone marrow of mice. The results of all these tests were negative. On the basis of these data, the Committee concluded that eprinomectin was unlikely to be genotoxic.

In a two-generation study of reproductive toxicity, rats were administered eprinomectin at dietary concentrations of 0, 6, 18 or 54 mg/kg of feed. On the basis of decreased food intake during the first 2 weeks of lactation, the NOEL for maternal toxicity was 18 mg/kg of feed, equal to 2.5 mg/kg of body weight per day. The NOEL for reproductive toxicity was 18 mg/kg of feed, equal to 1.6 mg/kg of body weight per day, based on delayed sexual maturation and reduced rate of pregnancy in the first-generation animals. Toxicity in pups was the most sensitive indicator of eprinomectin toxicity; decreased pup weights, body tremors and increased pup mortality were observed at a dietary concentration of 54 mg/kg of feed in the first-generation pups and in the second-generation pups of the first mating. Second-generation pups dosed at 18 mg/kg of feed also had body tremors. In the second-generation pups of the second mating, when intake of eprinomectin was reduced to 0, 3, 9 or 27 mg/kg of feed, no body tremors or mortality occurred at any dose level, but decreased pup weights were still evident at the highest dose. The NOEL for pup toxicity was 9 mg/kg of feed, equal to 1.3 mg/kg of body weight per day. It was further noted that no histopathological changes were observed in brain, spinal cord or sciatic nerves of animals dosed up to
38 weeks of age. A follow-up study suggested that the pup toxicity was most likely to be due to postnatal exposure through maternal milk, which contained high and sustained levels of eprinomectin when the drug was administered to the dams. This was further supported by a cross-fostering study with ivermectin, a closely related compound, which was reviewed by the Committee at its thirty-sixth meeting (Annex 1, reference 97).

In developmental toxicity studies with rats and rabbits, eprinomectin caused maternal toxicity. In rat dams, this was evident as changes in body-weight gain and food consumption at oral doses of 3 and 12 mg/kg of body weight per day, with a NOEL of 1 mg/kg of body weight per day. In rabbit dams, slowed pupillary reflex, mydriasis and decreased body-weight gain were observed at oral doses of 2 and 8 mg/kg of body weight per day, with a NOEL of 1.2 mg/kg of body weight per day. Oral administration of eprinomectin did not cause embryotoxicity, fetotoxicity or teratogenicity up to the highest doses tested in rats or rabbits (12 and 8 mg/kg of body weight per day, respectively).

No long-term toxicity studies were available on eprinomectin. However, data on long-term toxicity were available for emamectin, another amino-substituted avermectin structurally very similar to eprinomectin. The Committee noted that dogs were the most sensitive species to both emamectin and eprinomectin, and that the toxicological end-point for both compounds was neurodegenerative changes. It was further noted that, for both compounds, the neurotoxic effects did not progress upon prolonged treatment, resulting in the same NOELs in 90-day and 1-year studies in dogs. On the basis of this information, the Committee concluded that it was unnecessary to request data from long-term toxicity studies on eprinomectin.

Because the chemical structure of eprinomectin contains no structural features suggestive of carcinogenicity, and the avermectins emamectin and abamectin, which have closely related structures, were not carcinogenic in mice or rats, the Committee concluded that eprinomectin was unlikely to be carcinogenic. This conclusion was supported by the negative findings in in vitro and in vivo genotoxicity studies with eprinomectin.

The Committee considered that the most relevant effect for the safety evaluation of residues of eprinomectin was its effect on the mammalian nervous system. An ADI of 0–10 μg/kg of body weight was established, based on the NOEL of 1 mg/kg of body weight per day for mydriasis and focal neurodegenerative changes in the brain in the 1-year study in dogs, and a safety factor of 100.
Metabolism data

Groups of rats were given oral doses of [1,1,2,2,4C-5,3H] eprinomectin by gavage and killed at 24 hours and 48 hours after dosing. Less than 0.5% of the dose was excreted in the urine, and 76–99% of the administered dose appeared in the faeces within 48 hours of dosing. There was evidence of only minimal loss of the [3H] label as tritiated water. Eprinomectin B1a, was the major residue in both liver and faeces, accounting for 82% and 85%, respectively, of the total radioactivity after 24 hours and 73% and 80%, respectively, after 48 hours. At least five metabolites (M1–M5) were detected. The major metabolite in liver, M5 (N-deacetylleprinomectin), accounted for about 18% of the total residues at 48 hours.

In a related study, groups of rats received oral doses of approximately 6mg of [5,3H]eprinomectin B1a per kg of body weight by gavage for 7 consecutive days and were then killed at 7 hours, 1 day, 2 days and 5 days after the last dose. The predominant route of excretion was in the faeces, with urinary excretion accounting for less than 1% of the total radioactivity. The parent drug and all its radioactive metabolites could be extracted quantitatively with organic solvents from both tissues and faeces. Eprinomectin was the major residue in all tissues and plasma at 2 days after the last dose and in faeces at 4 days after the last dose. The metabolite M5 was the major metabolite in liver and kidney. The levels of total radioactivity were highest in the liver, fat and kidney, followed by the muscle and plasma and were similar in both male and female rats.

In the first of two studies with the radiolabelled drug in dairy cows, four lactating dairy cows were treated topically with [14C-5,3H]eprinomectin B1a, at a dose of 750µg/kg of body weight and killed 21 days after treatment. The maximum levels of total radioactivity in the faeces of two of the animals ranged from 820 to 3000µg/kg of eprinomectin equivalents. Approximately 32% of the dose was excreted in the faeces after 14 days and 34% after 21 days. More than 90% of the total radioactivity of the parent drug and its metabolites was extracted from liver, faeces and milk with ethyl acetate. Eprinomectin was metabolized only to a small extent in milk and in liver. It was the predominant residue and is therefore the appropriate marker residue. In a second study, four lactating dairy cows were treated topically with [5,3H]eprinomectin at a dose of 750µg/kg of body weight and killed 21 days after dosing. Only 0.32% of the radioactivity of the initial dose (estimated at approximately 1% of the bioavailable dose from other evidence) was excreted into milk during the first 14 days after treatment. In both of these studies, there was a close correlation between the metabolism of the drug and the
ratios of concentrations of total radioactive residues to those of eprinomectin $B_{1u}$. Significant variation in drug absorption and depletion between individual animals was reported in many studies on eprinomectin.

Twelve cattle of less than 1 year of age were treated with [5-$^3$H]eprinomectin in a single topical dose of 500$\mu$g/kg of body weight and killed in groups of three at 7, 14, 21 and 28 days after dosing. The maximum levels of radioactivity and eprinomectin $B_{1u}$ in plasma were 4.4–21$\mu$g/l and 7.3–20$\mu$g/l, respectively, and were attained 2–5 days after treatment. The major route of excretion was the faeces in which 14% of the total radioactivity was excreted compared to 0.35% in the urine of animals killed up to 28 days after dosing. Analysis of the skin of the cattle killed at 28 days after treatment showed that 54% of the radioactive dose remained in the skin, 89% of which was unmetabolized eprinomectin. More than 90% of the total radioactive residues from tissues, plasma and faeces could be extracted using organic solvent. The predominant residue in all tissue matrices at all times was the parent drug. This was accompanied by minor quantities of between 5 and 7 metabolites in tissues, plasma and faeces. Most of these metabolites accounted for about 1% or less of the total residues. The exceptions were metabolite M5 in muscle (3.9%) and metabolite M1 in faeces (7.4%). The Committee concluded that eprinomectin is not metabolized to any great extent in cattle tissues following topical application and that it is excreted, predominantly unchanged, in the faeces.

Residue data

 Twelve cattle of less than 1 year of age were treated with [5-$^3$H]eprinomectin in a single topical dose of 500$\mu$g/kg of body weight. Groups of three animals were killed at 7, 14, 21 and 28 days after dosing and total residue levels and levels of eprinomectin $B_{1u}$ residues were measured in muscle, liver, kidney and fat. The levels of total residues declined with a half-life of about 8 days in all tissues. The levels of total residues, determined by radioactivity, and eprinomectin $B_{1u}$, measured by HPLC, declined at a similar rate in all tissues at all sampling times. The ratio of eprinomectin $B_{1u}$ to total residues averaged over the four sampling times was 0.83, 0.85, 0.92, 0.71 and 0.69 in liver, kidney, fat, dosage-site muscle and muscle from elsewhere on the animal, respectively. On the basis of these studies, eprinomectin $B_{1u}$ is the marker residue.

In two separate studies, lactating cows were treated with radiolabelled eprinomectin at 1.5 times the recommended dose. In each study, total residues were estimated radiometrically and the
concentration of eprinomectin B$_{18}$ was determined by HPLC. In the first of these studies, four lactating dairy cows were treated topically with 750μg of [¹⁴C-5-³H]eprinomectin/kg of body weight and killed 21 days after treatment. The maximum levels of total radioactive residues in milk were reached within 7 days of dosing and were in the range of 8.3–26μg/l. The mean concentration of total radioactive residues and of eprinomectin B$_{18}$ in milk peaked 2 days after dosing. In the second study, four lactating dairy cows were treated and killed in the same manner as in the first study. Only 0.32% of the radioactive dose was excreted into milk up to 14 days after dosing. The maximum levels of total radioactive residues occurred within 7 days of dosing and were in the range of 3.1–9.0μg/l. The concentration of eprinomectin residues in milk peaked 3 days after treatment. In both studies, the ratio of marker residue to total radioactive residues was calculated at each sampling time. The average value, based on nearly 190 samples, was calculated to be 0.77 (range 0.67–0.87).

The levels of residues in tissues were determined at 21 days after treatment in the two studies in lactating cattle described above. In the first study, the average concentrations of total radioactive residues in liver, kidney, fat, dosage-site muscle and muscle from elsewhere on the animal were 120, 16, 8.6, 29 and 1.1μg/kg, respectively. The residue levels were consistently higher in two of the four cows and the elimination of drug in the milk of one cow was much faster and peaked at a much higher value than in the other three. In the second study, the average concentrations of total radioactive residues in liver, kidney, fat, dosage-site muscle and muscle from elsewhere on the animal were 146, 21, 12, 88 and 0.7μg/kg, respectively.

Six pregnant Holstein dairy cows were treated topically with [⁵⁻³H]eprinomectin 21 days prior to their anticipated delivery date. The eight resulting calves were killed 12–24 hours after birth. The residue levels were at or near the detection limits of the assay in all tissues except liver, where the levels of total residues averaged 21μg/kg (range 5.8–55μg/kg).

In an initial residue-depletion study using unlabelled eprinomectin, 30 beef cattle were treated topically with eprinomectin at 500μg/kg of body weight. Groups of five animals were slaughtered at 10, 17, 24, 34, 44 and 55 days after dosing. The levels of residues of eprinomectin B$_{18}$ were measured by HPLC. The recoveries for all tissues were 72–111% and the coefficients of variation of the analytical method were less than 10% in all tissues. At 10 days after treatment, the mean levels of eprinomectin residues in liver, kidney, fat, dosage-site muscle and muscle from elsewhere on the animal were 750, 75, 26, 8
and 6μg/kg, respectively, declining to 56, 9, 3, <2 and <2μg/kg, respectively, in the same tissues 24 hours after dosing.

In a second study with unlabelled eprinomectin, residue levels in the tissues were measured at earlier post-treatment times. Twenty-five beef cattle were treated topically with eprinomectin at 500μg/kg of body weight and killed (in groups of five) after 0.5, 1, 3, 5 and 7 days. The mean levels of eprinomectin in muscle, liver, kidney and fat reached a maximum at 3 days after dosing with values of 5, 710, 93 and 20μg/kg, respectively, declining to <2, 320, 23 and 4μg/kg, respectively, after 7 days.

A residue-depletion study was conducted in 12 non-ruminating male Holstein calves (less than 16 weeks old), which were treated topically with the recommended dose of eprinomectin (500μg/kg of body weight). Groups of three animals were killed at 1, 3, 7 and 14 days after dosing. The highest concentrations of the marker residue occurred 7 days after treatment in all tissues and were 48μg/kg in muscle, 1200μg/kg in liver, 240μg/kg in kidney and 290μg/kg in fat. The residues in the liver declined to a mean value of 800μg/kg after 14 days.

In a study designed to investigate the effect of eprinomectin residues on the production of yogurt and cheese, 30 milk-producing dairy cows were divided into three groups. Two groups were treated topically with eprinomectin at 500μg/kg of body weight, while the third group served as a control. Quantifiable amounts of eprinomectin B₁₄ were first detected 23 hours after treatment. Residues could be detected between 6 and 12 days after dosing. The maximum concentration of residues in milk was 11μg/l with a peak occurring after 2–3 days in 80% of the animals while in the remainder the peak in milk occurred 4–7 days after treatment. It was not possible to differentiate between yogurt and cheese produced from milk from treated and untreated animals in organoleptic trials. The levels of eprinomectin residues in milk were similar to those obtained in studies using radiolabelled eprinomectin.

**Analytical methods**

A high-performance liquid chromatography (HPLC) fluorescence method for the determination of eprinomectin residues in bovine tissues and milk has been developed. It is based on the formation of a strongly fluorescing aromatic derivative of eprinomectin. This derivative has a half-life of approximately 2 hours and is not as stable as the structurally analogous derivatives prepared from either abamectin or ivermectin. This necessitates the in-line preparation of
the derivative immediately prior to analysis by reversed-phase HPLC. Nevertheless, the analytical procedure is readily automated, which allows routine analyses to be performed overnight. The method, which uses an external standard, was thoroughly validated and can determine eprinomectin residues accurately over a wide concentration range (2–2500µg/kg). The limits of detection of the method were 1µg/kg for all tissues and 1µg/l for milk. The limits of quantification were 2µg/kg for all tissues and 2µg/l for milk. The coefficient of variation for all tissues was 12% (range 11–14%). There were no analytical interferences from related drugs.

A modification of the above method has been developed for milk using an internal standard that is an analogue of eprinomectin. Quantitative recovery of extracted residues was verified with radiolabelled eprinomectin and the lack of interference from both abamectin and ivermectin was established. The method was validated over a concentration range of 2–50µg/l and the limits of quantification and detection were determined to be 1.0 and 0.25µg/l, respectively.

**Maximum Residue Limits**

On the basis of an ADI of 0–10µg/kg of body weight for the parent drug established by the Committee, the permitted daily intake of the drug and/or its equivalents is 600µg for a 60-kg person. In recommending MRLs for eprinomectin, the Committee reviewed all four residue-depletion studies in cattle. It recognized that the residue-depletion data for one study in non-ruminating calves were different from those in the three studies in ruminating cattle. In particular, the levels of residues in muscle and fat in the study in calves were higher than those found in any of the three studies in cattle and required a reappraisal of the MRLs. Nevertheless, a set of MRLs that are consistent with all the available data can be recommended for all tissues and milk. In recommending MRLs for various tissue matrices, the Committee took the following factors into consideration:

- The drug is for use in dairy and beef cattle.
- The limits of quantification of the analytical methods are 1.0µg/l and 2.0µg/kg for milk and tissues, respectively.
- The marker residue is eprinomectin B₁a.
- The marker residue is always the predominant residue in both tissues and milk. Eprinomectin B₁a accounted for 69% of the total residues in muscle and 83% of those in liver; the corresponding values for kidney, fat and milk were 85%, 92% and 77%, respectively.
- The completeness of the total data set provided by the sponsor allowed values for MRLs in cows to be derived statistically.
Table 1

Theoretical maximum daily intake of eprinomectin residues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Recommended MRL (µg/kg)(^a)</th>
<th>Estimate of total residues (µg/kg)</th>
<th>Theoretical maximum daily intake(^b) (µg eprinomectin equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>100</td>
<td>145(^b)</td>
<td>44</td>
</tr>
<tr>
<td>Liver</td>
<td>2000</td>
<td>2410(^b)</td>
<td>241</td>
</tr>
<tr>
<td>Kidney</td>
<td>300</td>
<td>353(^b)</td>
<td>18</td>
</tr>
<tr>
<td>Fat</td>
<td>250</td>
<td>272(^b)</td>
<td>14</td>
</tr>
<tr>
<td>Milk</td>
<td>20(^a)</td>
<td>26(^h)</td>
<td>39</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>356</td>
</tr>
</tbody>
</table>

\(^a\) Expressed as eprinomectin equivalents.
\(^b\) Based on a daily intake of 0.5 kg of meat made up of 300 g of muscle, 100 g of liver, and 50 g each of kidney and fat and 1.5 litres of milk.
\(^c\) The marker residue accounted for 69% of the total residues in muscle.
\(^d\) The marker residue accounted for 83% of the total residues in liver.
\(^e\) The marker residue accounted for 85% of the total residues in kidney.
\(^f\) The marker residue accounted for 92% of the total residues in fat.
\(^g\) Expressed in µg/l.
\(^h\) The marker residue accounted for 77% of the total residues in milk.

- In recommending MRLs for eprinomectin, the Committee took into account the proportion of the total residues in all tissues over the total withdrawal times reported by the sponsor.
- The values for the proportion of the total residues accounted for by the marker residue used to set MRLs for eprinomectin in non-ruminating calves were those established in radiometric studies in cattle. The Committee concluded that the extent of metabolism of eprinomectin in calf tissues would probably be less than or equal to the extent of metabolism of the drug in the same tissues in cattle.

The Committee recommended MRLs for eprinomectin of 100 µg/kg for muscle, 2000 µg/kg for liver, 300 µg/kg for kidney, 250 µg/kg for fat and 20 µg/l for milk in cattle, expressed as eprinomectin equivalents.

From these MRLs, the theoretical maximum daily intake of eprinomectin residues would be 356 µg (Table 1).

3.1.2 Febantel, fenbendazole and oxfendazole

Febantel, fenbendazole and oxfendazole were previously evaluated at the thirty-eighth and forty-fifth meetings of the Committee (Annex 1, references 97 and 119).

Fenbendazole and oxfendazole are benzimidazoles, and are metabolically interconvertible in vivo. Febantel is a prodrug that can be converted in vivo by cyclization to fenbendazole or following oxidation at the sulfur atom and subsequent cyclization to oxfendazole.
At its forty-fifth meeting, the Committee established a group temporary ADI of 0-4μg/kg of body weight for febantel, fenbendazole and oxendazole, based on a NOEL of 0.7mg/kg of body weight per day for oxendazole identified in a 2-year study in rats and a safety factor of 200. At that time, the Committee requested the results of a teratogenicity study in rabbits in which oxendazole is administered at sufficiently high doses for its teratogenic potential to be adequately explored.

Toxicological data
At its present meeting, the Committee considered the results of new developmental toxicity studies using oxendazole in rabbits. The studies were conducted according to appropriate standards for study protocol and conduct. In three preliminary studies, doses of up to 1000mg/kg of body weight per day were given by gavage to pregnant rabbits during the critical days of gestation. Doses of 100mg/kg of body weight per day and higher produced fetal malformations. On the basis of the results from these studies, the compound was administered by gavage to pregnant rabbits on days 7 to 19 of gestation at doses of 0, 10, 30 or 45mg/kg of body weight per day. No maternal toxicity, embryotoxicity or effects on fetal morphology were observed in this study.

The Committee considered a published tumour initiation-promotion study with oxendazole in rats. In this study levels of P<sub>50</sub> enzymes, intercellular communication across gap junctions and expression of the placental form of glutathione S-transferase were examined. The Committee concluded that although some enzymatic and immunohistochemical changes suggested that oxendazole might have tumour-promoting potential in rats, results from the studies on genotoxicity and carcinogenicity evaluated at the thirty-eighth and forty-fifth meetings presented sufficient evidence that oxendazole is not carcinogenic.

The Committee established a group ADI of 0-7μg/kg of body weight for febantel, fenbendazole and oxendazole, based on the NOEL of 0.7mg/kg of body weight per day for oxendazole identified in the 2-year study in rats evaluated at the thirty-eighth and forty-fifth meetings and a safety factor of 100.

The Committee noted that this ADI provides a safety factor of 1000 for teratogenic effects in sheep, which was considered to be the species most sensitive to the teratogenic effects of oxendazole, as evaluated at the thirty-eighth meeting.
Metabolism data
The oxidative metabolism of fenbendazole was studied in vitro using liver tissue from a number of species, including cattle, sheep and goats. The sulfoxide metabolite (oxfendazole) and, upon further oxidation, the sulfone (oxfendazole sulfone), were produced in the preparations of liver from all the species investigated, but at varying rates. Although some degree of species-specific variation in the rates of formation of the two principal metabolites was seen, the differences were not of practical significance.

The distribution of fenbendazole was studied in the plasma, urine and faeces of goats after oral and intravenous administration. Fenbendazole, oxfendazole and oxfendazole sulfone were found to be the major residues in plasma, as in other species, including cattle and sheep.

Pharmacokinetic data
The pharmacokinetics of oxfendazole in goats and sheep was compared. After intravenous administration of 7.5 mg of oxfendazole/kg of body weight to sheep and goats, the areas under the concentration–time curves (AUCs) for the two species were not significantly different. Similarly, the total AUCs for the three metabolites were not significantly different. However, the bioavailability of oxfendazole after oral administration in goats was only about 42% of that in sheep.

The pharmacokinetics of fenbendazole administered as a 4% powder formulation or a 1.5% pellet formulation at a dose equal to 5 mg/kg of body weight was determined in a study in 12 pigs. The mean pharmacokinetic parameters of all pigs were compared after administration of either the pellets or powder. The maximum concentrations, the times for maximum concentrations to be reached and the AUCs were similar for the two formulations.

Residue data
At its thirty-eighth meeting, the Committee recommended group MRLs for febantel, fenbendazole and oxfendazole using oxfendazole sulfone as the marker residue. Temporary MRLs, expressed as the sum of the three principal metabolites (fenbendazole, oxfendazole and oxfendazole sulfone) calculated as oxfendazole sulfone equivalents, were recommended for cattle, sheep and pigs: 100 μg/kg for muscle, fat and kidney, 500 μg/kg for liver and 100 μg/l for milk (cattle and sheep).
At its forty-fifth meeting, the Committee reviewed several residue-depletion studies in cattle, sheep and pigs. However, the residue-depletion studies on total residues of fenbendazole, oxendazole and oxendazole sulfone in cattle and sheep following the administration of febantel and oxendazole were still underway. In addition to the results from these studies, the Committee noted that, with the increasing production of goats in developing countries, residue data would be required for establishing MRLs in this species.

At its present meeting, the Committee reviewed the results of the residue-depletion studies on febantel and oxendazole in cattle and sheep as well as three new studies with fenbendazole, including one study in horses and two studies in pigs.

**Cattle.** A residue-depletion study was conducted in cattle, using a 10% suspension of febantel administered orally at a dose of 7.5 mg/kg of body weight. The cattle were killed in groups (four per group) 7, 14, 21 and 28 days after treatment. The concentrations of oxendazole sulfone in muscle and kidney were at or below the limit of quantification at all times. The concentrations in liver and fat were 115 and 19 µg/kg, respectively, at day 7 and were at or below the limit of quantification at all other times.

Three further residue-depletion studies were conducted in cattle. In the first study, 32 cattle were administered single oral doses of an oxendazole suspension at 4.5 mg/kg of body weight. Groups of cattle (four per group) were slaughtered 10, 12, 14, 16, 18, 20, 22 and 24 days after treatment. No residues of oxendazole or its metabolites were detectable in kidney and muscle after day 10. The mean levels of metabolites present in liver were less than 20 µg/kg by day 14 and below 10 µg/kg by day 18. The levels in fat were less than 10 µg/kg on day 10 and were no longer detectable by day 14.

In the second study, eight lactating cows were given single oral doses of an oxendazole suspension at 4.5 mg/kg of body weight. Milk samples were collected from each cow immediately prior to treatment and every 12 hours thereafter up to 120 hours after treatment. The levels of residues of oxendazole and its metabolites peaked at 220 µg/l at 24 hours after treatment and declined to near or below the limit of quantification at 96 hours.

In the third study, single doses of a 10% suspension of febantel were administered orally to eight lactating cows at 7.5 mg/kg of body weight. Milk samples were taken from 12 animals (four of them were untreated) beginning 3 days before and continuing until 5 days after administration of febantel. Two samples were collected from each
cow per day, one in the morning and one in the evening. The levels of residues of febantel and its metabolites reached a maximum of 268μg/l at 34 hours after administration and were near the limit of quantification at 96 hours.

**Sheep.** Single doses of a 2.5% suspension of febantel were administered orally to 16 sheep at 5.0mg/kg of body weight. Groups of four animals were killed 3, 7, 14 and 21 days after treatment. The concentrations of residues of febantel and its metabolites in muscle, liver, kidney and fat were 40, 4600, 200 and 130μg/kg, respectively, at 3 days after administration. The levels of residues had declined to near or below the limit of quantification in muscle, liver, kidney and fat by days 7, 21, 14 and 14, respectively.

A 2.3% suspension of oxfendazole was administered as a single oral dose to 36 sheep at 5.9mg/kg of body weight. The sheep were killed in groups of two treated males and two treated females 12, 14, 16, 18, 20, 22 and 24 days after treatment. The concentrations of residues of oxfendazole sulfone were below the limit of quantification in muscle, kidney and fat at all sampling times. Liver contained 480, 290 and 130μg/kg of oxfendazole sulfone 10, 12 and 14 days after treatment, respectively. At all later times, the concentrations were at or below the limit of quantification.

In a study conducted in lactating sheep, a 2.5% suspension of febantel was administered orally as a single dose to eight lactating sheep at 5.0mg/kg of body weight. Milk samples were taken from the animals beginning 3 days before and continuing until 5 days after administration. Two samples were collected from each sheep per day, one in the morning and one in the afternoon. The concentrations of residues peaked at 360μg/l at 10 hours after administration and declined to the limit of quantification by 106 hours.

**Goats.** Two groups of four goats each were dosed orally with a suspension of fenbendazole at 5mg/kg of body weight (the recommended dose) and 25mg/kg of body weight. The concentration of fenbendazole was determined in milk at 2 hours after treatment and then at 12-hourly intervals until 120 hours after treatment. The detection limit of the method was 0.01mg/l. Although the metabolites of fenbendazole were also observed on the chromatograms, they were not quantified. The highest levels, 98μg/l and 443μg/l, were observed 12 hours following administration of the 5 and 25mg/kg of body weight doses, respectively. The levels of fenbendazole were below the limit of detection 48 hours after administration of the 5 mg/kg of body weight dose and 72 hours after administration of the 25 mg/kg of body
weight dose. Fenbendazole was eliminated from the milk with a half-life of 9.6 hours.

**Pigs.** Five groups of pigs (five per group) were treated orally with a 1.5% pellet formulation of fenbendazole at a dose of 5mg/kg of body weight. The levels of combined residues, expressed as oxfendazole sulfone, were determined in muscle, liver, kidney, fat and skin 12, 24, 72, 120 and 168 hours after treatment. The concentrations of residues of fenbendazole and its metabolites peaked at 920µg/kg in muscle, 6300µg/kg in liver and 100µg/kg in kidney, 24 hours after dosing. The concentrations of residues in skin and fat peaked at 980 and 1300µg/kg, respectively, 12 hours after treatment.

**Horses.** A study was conducted in horses using a 10% suspension of fenbendazole administered orally to 16 horses at a dose of approximately 10mg/kg of body weight daily for 5 consecutive days. The horses were slaughtered in groups of two males and two females 5, 10, 15 and 20 days after the last treatment and tissue samples were taken for analyses. The analyses showed that by 5 days after the final treatment, the levels of fenbendazole and its metabolites had declined below the limit of quantification.

**Analytical method**
An analytical method is available for the quantitative determination of residues of fenbendazole and its metabolites (oxfendazole and oxfendazole sulfone) in the edible tissues of cattle, sheep, pigs and horses and in milk (cattle and sheep). The method can also be applied to the determination of fenbendazole and its metabolites in the skin of pigs.

Fenbendazole and its two metabolites were extracted from milk and tissue homogenates using ethyl acetate. Fenbendazole and oxfendazole were oxidized to oxfendazole sulfone with peracetic acid. The total amount of oxfendazole sulfone was analysed quantitatively after extensive purification using HPLC with fluorescence detection. An internal standard was used to correct for recoveries. The method had a linear range of 5–1000µg/kg in liver and 5–200µg/kg in kidney, fat and muscle of all species investigated, as well as in skin from pigs. The linear range in milk from cattle and sheep was 5–1000µg/l.

On the basis of a statistical evaluation of the data on the precision of the method made by the Committee for various tissues and milk in different species, the limit of quantification was found to vary from approximately 5 to 35µg/kg.
Maximum Residue Limits

In reaching its decision on MRLs for febantel, fenbendazole and oxfendazole, the Committee took into account the following factors:

- An ADI of 0–7μg per kg of body weight was established. This would result in a maximum ADI of 420μg for a 60-kg human.
- The data on metabolism, pharmacokinetics and residue depletion are similar in cattle, sheep, goats, pigs and horses.
- The correlation between residues in plasma and milk is similar in sheep and cows.
- The data on analytical performance provided by the sponsor show that the highest limit of quantification for any of the edible tissues or milk is well below the recommended MRLs.
- The residues in tissues and milk in all species are calculated as oxfendazole sulfone equivalents.
- The MRLs are expressed as the sum of the three principal metabolites (fenbendazole, oxfendazole and oxfendazole sulfone), calculated as oxfendazole sulfone equivalents.

The Committee recommended MRLs for febantel, fenbendazole and oxfendazole of 100μg/kg for muscle, kidney and fat and 500μg/kg for liver in cattle, horses, pigs, sheep and goats. The Committee also recommended an MRL of 100μg/l for milk in cattle and sheep. These MRLs would result in a maximum daily intake of 240μg of residues of febantel, fenbendazole and oxfendazole, based on a daily food intake of 300g of muscle, 100g of liver, 50g each of kidney and fat, and 1.51 of milk.

3.1.3 Moxidectin

Moxidectin is a macrocyclic lactone that is used to control a range of internal and external parasites in sheep, cattle and deer. It was previously evaluated at the forty-fifth, forty-seventh and forty-eighth meetings of the Committee (Annex 1, references 119, 125 and 128). At its forty-fifth meeting, the Committee established an ADI of 0–2μg/kg of body weight and recommended MRLs of 20μg/kg for muscle, 100μg/kg for liver, 50μg/kg for kidney and 500μg/kg for fat, expressed as parent drug, in cattle, sheep and deer. The MRLs for deer were temporary, pending the receipt of further information on the marker residue in the edible tissues, which was required for evaluation in 1998. At its forty-seventh meeting, the Committee recommended that the MRL for sheep muscle be increased to 50μg/kg.

Residue data

The data available at the forty-fifth meeting of the Committee clearly demonstrated that moxidectin is the marker residue in the edible
tissues of cattle and sheep. However, it was not possible to re-commend definitive MRLs for moxidectin in deer because its metabolism in deer was not known and the relationship between moxidectin and total residues was also unknown. At its present meeting, the Committee reviewed the results of in vitro studies conducted to address these questions.

A liver microsome assay with [14C]moxidectin was employed to de-scibe and compare the metabolic profiles obtained for moxidectin in liver preparations from various animal species. Livers of deer, cattle, sheep and goats were collected from four animals of each species and microsomes were prepared. The microsomes were incubated with [14C]moxidectin at 37–38°C. The incubates were extracted and analysed by HPLC. The results of the liver microsome studies indicated that moxidectin was the main component of the extract follow-ing incubation, accounting for 70%, 65% and 69% of the total radioactivity in the microsomal preparations from cattle, sheep and deer, respectively. The chromatographic profile of the other metaboli-tes was similar for each species with only minor differences observed between cattle, sheep and deer. For preparations from deer liver, no moxidectin metabolite accounted for more than 10% of the total radioactivity. These results indicated that moxidectin metabolism in deer is comparable to that in cattle and sheep and moxidectin should be the marker residue in all three species.

Residue data from a study of moxidectin in red deer were presented at the forty-fifth meeting of the Committee. Twenty deer, aged 15–16 months, were treated with a pour-on preparation of moxidectin at a dose of 0.5 mg/kg of body weight. Groups of five animals were killed at 7, 14, 21 and 28 days after treatment. Edible tissues were collected and the moxidectin residues assayed. At all sampling times the resi-dues were below the limit of quantification: <24 μg/kg in muscle, <6 μg/kg in liver and <11 μg/kg in kidney. The mean values (and upper 99% confidence limits) in fat were 130 μg/kg (270 μg/kg) at 7 days, 160 μg/kg (230 μg/kg) at 14 days, 57 μg/kg (180 μg/kg) at 21 days and 31 μg/kg (140 μg/kg) at 28 days. These values were less than the pro-posed MRLs at all sampling times.

**Maximum Residue Limits**

At the forty-fifth meeting, an ADI of 0–2 μg/kg, equivalent to 120 μg per day for a 60-kg person, was established. At that meeting, the Committee recommended MRLs for cattle and sheep and temporary MRLs for deer of 20 μg/kg in muscle, 100 μg/kg in liver, 50 μg/kg in kidney and 500 μg/kg in fat, expressed as parent drug, based upon the following factors:
• Fat and liver are the target tissues.
• The parent drug is the marker residue and accounts for 40% of the total residues in muscle, liver and kidney and 75% of the total residues in fat.
• Bound residues account for 5–15% of the total residues, and information was not available to enable them to be disregarded in the calculation of the MRLs.
• The quantification limit of the analytical method (10μg/kg).
• The sponsors do not propose to make the drug available for use in lactating cows and cows in late pregnancy. Thus, residues in milk should not be taken into account.

At its present meeting, the Committee confirmed the MRLs for moxidectin in deer and removed the temporary qualification.

3.2 Antimicrobial agents

The Committee examined the results of in vitro studies of antimicrobial activity using relevant human gut microflora to derive ADIs for some of the antimicrobial agents that were reviewed. The equation used for deriving these ADIs is based on that described in the report of the forty-seventh meeting of the Committee (Annex 1, reference 125).

The equation is as follows:

\[
\frac{\text{Upper limit of ADI (μg/kg of body weight)}}{\text{MIC}_{90}(μg/g)} = \frac{\text{Mass of colonic contents (g)}}{\text{Fraction of oral dose} \times \text{Safety factor} \times \text{Weight of human (kg)}} \times \text{Bioavailability}
\]

where:

\( \text{MIC}_{90} \) = Minimum concentration of an antimicrobial drug completely inhibiting the growth of 50% of the cultures of a particular microorganism, as judged by the naked eye, after a given period of incubation. For the purpose of the evaluation, the \( \text{MIC}_{90} \) value is the mean \( \text{MIC}_{90} \) for the strain(s) of the relevant species tested. Alternatively, the lowest \( \text{MIC}_{90} \) value for the most sensitive species can be used.

A value of 220g is used for the mass of the colonic contents and a value of 60kg is used for the weight of an adult. The safety factor used

\[1\text{ Although MIC}_{90} \text{ values are usually expressed in μg/ml, they are expressed as μg/g in this equation so that the ADI will be in μg/kg. When the MIC}_{90} \text{ value is converted to these units, it is assumed that the density of the experimental medium is 1 g/ml.}\]
to take account of uncertainty about the amount and relevance of data available for review may range from 1 to 10. A value of 1 is used when extensive relevant microbiological data are provided.

3.2.1 Gentamicin

Gentamicin, an aminoglycoside antibiotic, was evaluated by the Committee at its forty-third meeting (Annex 1, reference 113), when a temporary ADI of 0–4 μg/kg of body weight was established. At that time, the Committee recommended temporary MRLs 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 cows’ milk, all of the values being expressed as parent drug. At its forty-third meeting, the Committee requested the following information for evaluation in 1997:

1. The results of studies on the effects of gentamicin on specific genera of microorganisms obtained from the human intestine.
2. Additional data to assist in the assessment of carcinogenic potential, which should include:
   (a) results of genotoxicity assays for gene mutations in mammalian cells and chromosomal aberrations in vitro and in vivo; and
   (b) details of an investigation on possible structural similarities between gentamicin and known carcinogens.
3. A validated chemical analytical method with a limit of quantification below the MRL recommended for milk.

Gentamicin was on the agenda for evaluation at the forty-eighth meeting of the Committee (Annex 1, reference 128). However, the results of these investigations were not available at that time. The Committee was informed that the necessary studies were under way and that the results would be available in the near future. Therefore, the Committee extended the temporary ADI and temporary MRLs for gentamicin until 1998.

Toxicological data

At its present meeting, the Committee considered the results of in vitro and in vivo mutagenicity studies, structural similarities of gentamicin to known carcinogens and the effects of gentamicin on specific bacterial species obtained from the human gut. The studies were carried out according to appropriate standards for study protocol and conduct.

Gentamicin was tested in vitro for its ability to cause gene mutations and chromosomal aberrations in Chinese hamster ovary cells and in vivo for its ability to induce nuclear anomalies in bone marrow cells of
mice when administered intravenously at dose levels of 20 to 80 mg/kg of body weight. The results of these tests were negative and the Committee concluded that gentamicin is unlikely to be genotoxic.

An analysis of possible structural similarities between gentamicin and known carcinogens was performed. None of the structural features listed in the available databases for carcinogenicity were found within the chemical structure of gentamicin. In view of this, and since the aminoglycosides that have been tested (neomycin, dihydrostreptomycin and paromomycin) do not elicit a tumorigenic effect in rats, the Committee considered a possible carcinogenic activity of gentamicin unlikely. This conclusion was supported by the negative results for genotoxicity in mammalian cells in in vitro and in vivo assays.

**Microbiological data**

The effect of gentamicin on the growth of 110 bacterial strains obtained from the human gastrointestinal flora was evaluated after incubation in vitro. The minimum gentamicin concentration resulting in 50% inhibition (MIC$_{50}$) was determined for 80 isolates of the eight predominant groups of anaerobic microflora in the human intestine. These included *Bacteroides* spp., *Lactobacillus* spp., *Bifidobacterium* spp., *Prevotella* spp., *Eubacterium* spp., *Clostridium* spp., *Fusobacteria* spp. and anaerobic Gram-positive cocci. In addition, data were provided for 30 isolates of the facultative anaerobes *Enterococcus* spp., *Proteus* spp. and coliforms. The MIC values for all 110 isolates ranged from 0.04 to >128 µg/ml. Although the facultative anaerobic bacteria were the most sensitive organisms, the Committee agreed that they should not be used in the calculation of the ADI because they are not predominant species in the human intestine. Instead, the Committee derived the ADI from data available on the most sensitive relevant genus isolated from the human gastrointestinal tract, in this case *Eubacterium* spp. The geometric mean MIC$_{50}$ for this genus was 6 µg/ml at an inoculum density of 10$^6$ colony-forming units per ml. *Eubacterium* spp. were also used in the evaluation at the forty-third meeting of the Committee, when an MIC$_{50}$ value of 0.8 µg/ml was used for establishing the temporary ADI. Although the MIC$_{50}$ values identified at the forty-third and the present meeting were different, the Committee concluded that the value obtained using isolates from the human gut flora was more relevant for establishing an ADI.

In calculating an ADI based on the antimicrobial activity of gentamicin, the Committee used the formula described in section 3.2:
Upper limit of ADI = \( \frac{6 \mu g/l \times 220 g}{1 \times 1 \times 60 kg^2} = 22 \mu g/kg \) of body weight

The Committee established an ADI of 0–20 \( \mu g/kg \) of body weight for gentamicin, based on the microbiological end-point. The Committee noted that the lowest NOEL identified at its forty-third meeting based on toxicological studies was 10 mg/kg of body weight per day, which is 500 times the ADI based on a microbiological end-point.

**Analytical methods**

The residue studies considered at the forty-third meeting of the Committee relied primarily on assays of microbial growth inhibition. Given the non-specificity of such assays and the availability of liquid chromatography assays for gentamicin residues in edible tissues, the Committee requested that a validated chemical analytical method be provided for the analysis of gentamicin residues in milk, with a limit of quantification below the MRL.

At its forty-third meeting, the Committee noted that, while no analytical methods were available that met the multi-laboratory validation criteria of the Codex Committee on Residues of Veterinary Drugs in Foods (5), there were methods published in the current scientific literature for the analysis of gentamicin residues in edible tissues based on HPLC. Several such methods were included in the compilations of analytical methods prepared for regulatory authorities.

An HPLC method for the quantification of residues of gentamicin in cows’ milk, as well as in muscle, liver, kidney and fat of cattle and pigs and skin of pigs was reviewed. The method for analysis of tissues, except fat, includes solvent extraction of the analyte into buffer, deproteination by heating, purification using solid-phase extraction and analysis by liquid chromatography. Detection is by fluorescence after pre-column derivatization. Deproteination is not required for the analysis of fat or skin from pigs, and the initial extraction of the analyte into buffer is omitted for the analysis of cows’ milk. The four major components of gentamicin (C1, C1a, C2 and C3a) are separated, with retention times of 20–25 minutes, and quantification is by com-

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1. For the purpose of this evaluation, the MIC\(_{90}\) value is the geometric mean MIC\(_{90}\) for gentamicin against the 10 strains of the most sensitive relevant genus isolated from the human intestinal tract, in this case *Eubacterium* spp.
2. The mass of the colonic contents.
3. Absorption of gentamicin following oral administration is poor; therefore, a factor of 1 was used to represent 100% availability in the gastrointestinal tract.
4. A safety factor of 1 was used because sufficient relevant microbiological data were available.
5. The weight of an adult person.
comparison with an external standard. Interference prevents the use of all four components for quantitative analysis in some tissue matrices. A standard containing the four major components was used in this study. For quantification, the Committee considered that well-characterized standards, preferably of each of the four major gentamicin components, should be used for calibration in the analysis. The method was tested using samples fortified at the MRLs recommended by the Committee at its forty-third meeting. The recoveries were 72–97% for tissues and 71% for cows' milk. The method appears to be suitable for use in a regulatory programme to determine compliance with recommended MRLs for residues in edible tissues from cattle and pigs, and in cows' milk.

Maximum Residue Limits
In recommending MRLs for gentamicin, the Committee took into account the following factors:

- An ADI of 0–20μg/kg of body weight, based on a microbiological end-point, was established. This corresponds to a maximum ADI of 1200μg for a 60-kg person.
- Gentamicin residues persist in kidney and liver, but deplete rapidly in muscle, fat and milk. Kidney and liver are therefore considered to be the appropriate target tissues.
- A suitable analytical method is available for measurement of gentamicin residues in edible tissues and milk. The limits of quantification were 100μg/kg for muscle and fat, 200μg/kg for liver, 1000μg/kg for kidney and 100μg/l for milk.
- The parent drug is the marker residue.

On the basis of the maximum levels of residues observed in studies with gentamicin in food animals reviewed at the forty-third meeting, the Committee recommended MRLs for gentamicin of 100μg/kg for muscle and fat, 2000μg/kg for liver and 5000μg/kg for kidney in cattle and pigs, expressed as parent drug. The Committee also recommended an MRL of 200μg/l for cows' milk, expressed as parent drug.

The MRLs recommended above would result in a theoretical maximum daily intake of 785μg, based on a daily food intake of 300 g of muscle, 100 g of liver, 50 g each of kidney and fat and 1.5 l of milk.

3.2.2 Procaine benzylpenicillin
Penicillins are a class of β-lactam antibiotics that are active primarily against Gram-positive bacteria. They are widely used in both human and veterinary medicine.
Procaine benzylpenicillin is the procaine ester of benzylpenicillin. Benzylpenicillin had been previously evaluated at the twelfth and thirty-sixth meetings of the Committee (Annex 1, references 17 and 91). However, neither procaine benzylpenicillin nor procaine has been evaluated previously.

Although toxicological studies specific to procaine benzylpenicillin were not available, data were available on disposition of the procaine moiety. Procaine has been one of the most widely used local anaesthetics in human medicine. Numerous hydrolytic enzymes are found in plasma and other tissues that rapidly hydrolyse procaine to p-aminobenzoic acid and diethylaminoethanol (6). Similarly, following oral exposure to procaine benzylpenicillin, acid hydrolysis of the procaine ester moiety would be expected to proceed rapidly, yielding p-aminobenzoic acid. Procaine and its hydrolysis products are generally considered to be non-toxic (7).

Because of the general lack of toxicity, rapid metabolism and widespread use of the procaine moiety of procaine benzylpenicillin, the Committee considered residues of procaine benzylpenicillin to be equivalent to residues of benzylpenicillin. The most prevalent adverse reactions reported in humans consuming food containing benzylpenicillin residues are allergic reactions in sensitive individuals. At its thirty-sixth meeting, the Committee recommended that the daily intake of penicillin from food be kept as low as practicable, and in any case, below 30μg of the parent drug. As with benzylpenicillin, the Committee concluded that allergy was the determining factor in the safety evaluation of residues of procaine benzylpenicillin.

The Committee concluded that daily intake of residues of benzylpenicillin and procaine benzylpenicillin should be kept below 30μg of the penicillin moiety.

**Pharmacokinetic data**

No data were available for review on studies with laboratory animals or on studies using radiolabelled procaine benzylpenicillin. However, the Committee noted that a number of studies that did not comply with the requirements for good laboratory practice had been carried out on food animals since benzylpenicillin was last reviewed, some of which had been conducted by regulatory authorities. The results of these studies were reviewed at the present meeting.

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¹ In the studies described here, 1 mg of procaine benzylpenicillin is equivalent to 1667 International Units (IU) of the drug.
Five calves received procaine benzylpenicillin as a single intramuscular injection at a dose of approximately 18000 IU/kg of body weight. The maximum concentration in plasma was 2.1 IU/ml, with an elimination half-life of 4.3 hours and AUC of 18 IU/ml × h. The decline in benzylpenicillin concentrations in plasma was monophasic.

Six calves received procaine benzylpenicillin at 30 mg/kg of body weight (approximately 30000 IU/kg of body weight) by intramuscular injection in the neck. The maximum concentration was 5.4 μg/ml in serum at 1.5 hours after injection and 1.5 μg/ml in tissue-compartment fluid at 7.7 hours. The elimination half-life was 3.0 hours in serum and 10 hours in tissue-compartment fluid.

Six cattle received intramuscular injections of procaine benzylpenicillin at 10000 IU/kg of body weight, in combination with dihydrostreptomycin (12.5 mg/kg of body weight). The procaine benzylpenicillin was eliminated in the serum with a half-life of 1.9 hours. Six mature cows received an intramuscular injection of procaine benzylpenicillin at 20000 IU/kg of body weight and 7 days later the same dose was administered by subcutaneous injection, repeated on 3 successive days. The maximum concentration in serum was found 30 min after intramuscular injection and 2 hours after subcutaneous injection. The concentration of benzylpenicillin in plasma decreased more rapidly in the first 24 hours following subcutaneous injection than after intramuscular injection. The residues of benzylpenicillin in the tissues of the animals that were killed 5 days after the final subcutaneous injection were distributed as follows: 0.13 mg/kg in muscle (diaphragm), 0.10 mg/kg in muscle (gluteal), 1.0 mg/kg in liver, 0.90 mg/kg in kidney (renal cortex), 0.58 mg/kg in kidney (renal medulla), 0.06 mg/kg in fat and 1.2 mg/kg in muscle adjacent to the injection site.

In a study in steers, procaine benzylpenicillin was administered intramuscularly as a single dose of up to 66000 IU/kg of body weight (one group) or as five successive daily doses (two groups) or subcutaneously as a single dose (one group). The highest maximum concentration in plasma, 4.2 μg/ml, was observed after intramuscular injection of a single dose in the neck. This treatment also provided the shortest plasma elimination half-life of 8.8 h. The maximum concentration in plasma occurred within 5–6 hours after injection in all treatment groups.

The pharmacokinetics of procaine benzylpenicillin used in combination with benzathine benzylpenicillin (1:1), administered intramuscularly or subcutaneously to yearling steers, was compared with intramuscular injection of benzathine benzylpenicillin alone. The
maximum concentration in plasma was observed within 1–4 hours after treatment with the combined formulation, and the half-life was 41–58 hours for all intramuscular injections. For the subcutaneous injection of the combined formulation, the half-life was 29 hours. The AUC varied with the dose and mode of injection, from 0.17 μg/ml × h for the subcutaneous injection to 0.35–0.97 μg/ml × h for intramuscular injection of the combined formulation and 0.65 μg/ml × h for benzathine benzylpenicillin alone. The results demonstrated the variability in pharmacokinetic parameters associated with the use of different formulations, and with intramuscular or subcutaneous injection of the same product.

In a study in nine calves, three different commercially available formulations containing procaine benzylpenicillin (200000 IU/ml) and dihydrostreptomycin (150–200 mg/ml) were administered at a dose of 0.1 ml/kg of body weight by intramuscular injection. No statistical difference was observed in the AUC \( \text{AUC}_{0-4} \) (mean 13.2–13.4 μg/ml × h) or AUC \( \text{AUC}_{0-\infty} \) (mean 13.7–14.0 μg/ml × h) in serum samples collected from 0.5 to 72 hours after treatment. There were significant differences in the half-life, which varied from 5.5 to 8.3 hours, and in the maximum concentration in plasma, which ranged from 1.1 to 1.5 μg/ml.

In a study in horses, procaine benzylpenicillin was injected intramuscularly or subcutaneously in different muscle groups at 20000 IU/kg of body weight. The highest AUC \( \text{AUC}_{0-12h} \) (26 IU/ml × h) and shortest half-life (8.0 hours) were observed following injection in the front shoulder. Six ponies were given procaine benzylpenicillin intramuscularly at 12 mg/kg (equivalent to 12000 IU/kg) of body weight. The resulting AUC was 8.8 μg/ml × h for plasma and 4.8 μg/ml × h for tissue-compartment fluid; maximum concentrations were reached within 3.5 hours and 12 hours, respectively. The renal elimination of benzylpenicillin was rapid, with a maximum residence time of 10 hours for benzylpenicillin in plasma, while the concentrations in tissue-compartment fluid exceeded those in the plasma only after the maximum concentration in the plasma had been reached and concentrations in both compartments were decreasing.

The difference in pharmacokinetics of procaine benzylpenicillin between healthy and sick animals was demonstrated in a study in Pasteurella multocida-free and infected rabbits, which received single intramuscular injections of procaine benzylpenicillin at 60000 IU/kg of body weight. Higher concentrations of benzylpenicillin were found in blood samples collected from the infected rabbits than from the non-infected rabbits up to 8 hours following treatment. In infected rabbits, the maximum concentration in the serum was 5.9 μg/ml at
1 hour, declining to 1.9μg/ml at 8 hours, while in the non-infected rabbits the maximum concentration was 2.3μg/ml at 3 hours, declining to 0.53μg/ml at 8 hours. In another study in rabbits, three commercial products containing procaine benzylpenicillin and dihydrostreptomycin were administered in an experiment similar to the study in calves. The half-life varied from 110 to 370 minutes and the maximum concentration in serum decreased from 4.4 to 2.1μg/ml as the elimination half-life increased, demonstrating again that different formulations result in different elimination profiles.

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

Residue data

Procaine benzylpenicillin is one of a number of available formulations of benzylpenicillin. At its thirty-sixth meeting, the Committee recommended MRLs of 50μg/kg for muscle, liver and kidney (all species) and 4μg/kg for milk (Annex 1, reference 97). The Committee also noted the limited availability of chemical assay methods and recommended the following:

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

At its present meeting, the Committee reviewed the results of a number of new residue-depletion studies, including two studies in cattle and pigs and one study in broiler chicks.

A study was conducted in which yearling steers received five successive daily intramuscular injections of procaine benzylpenicillin at 24000 or 66000IU/kg of body weight, or five successive daily subcutaneous injections of 66000IU/kg of body weight. Another group of steers received procaine benzylpenicillin subcutaneously at 66000IU/kg of body weight at different sites 10, 15, 20 and 30 days before slaughter. The tissues were analysed for benzylpenicillin residues using a liquid chromatography method with a detection limit of 0.005mg/kg. The mean residue levels were<0.05mg/kg in all tissues at 4 days after the final injection in the group treated at 24000IU/kg of body weight intramuscularly, while 10 days were required to reach this concentration range in the group given 66000IU/kg of body weight intramuscularly. The residues were more persistent following
subcutaneous administration of procaine benzylpenicillin. The distribution of residues was as follows at 4 days after the final dose: 0.48mg/kg in liver, 0.39mg/kg in kidney, 0.013mg/kg in diaphragm muscle, and <0.005mg/kg in gluteal muscle. The residue levels were <0.005mg/kg in many of the samples of injection-site tissue collected from animals treated intramuscularly at 24000IU/kg of body weight and were <0.005-1.2mg/kg in samples from animals given 66000IU/kg of body weight intramuscularly. While there was no clear relationship between time from treatment to slaughter and residue levels found at the injection site following intramuscular administration of procaine benzylpenicillin, several instances of entrapment of the drug in the intramuscular injection site were noted. One injection-site sample had residue levels of 1.2mg/kg at 10 days after the final treatment, while another contained 0.44mg/kg at 16 days after the final dose. These injection sites would not have been readily detected in a routine postmortem inspection. The high residue levels were attributed to the injection of volumes in excess of 30ml of the formulated product. Administration of the drug by subcutaneous injection resulted in visible deposits of the drug at the injection site at slaughter; the area was characterized by edema and haemorrhage. When injection-site tissue was excluded, the highest residue levels were found in the liver, followed by kidney and muscle, after both intramuscular and subcutaneous injection.

In a subsequent study, cattle were given either a combination of procaine benzylpenicillin and benzathine benzylpenicillin (1:1) intramuscularly or subcutaneously at the recommended dose (8600 and 8800IU/kg of body weight, respectively), or benzathine benzylpenicillin alone intramuscularly at 12000IU/kg of body weight. Residues were detectable in liver (0.007mg/kg) 14 days after intramuscular treatment with the mixed penicillins and at 30 days following subcutaneous administration (0.013mg/kg). The residue levels in the intramuscular injection sites were 3.2mg/kg at 14 days after treatment and 2.1mg/kg at 30 days, while residue levels at the subcutaneous injection sites were 1.6mg/kg at 30 days. The mixed penicillins were also given intramuscularly at 24000IU/kg; the resulting residue levels in the injection sites were 100mg/kg at 14 days after treatment and 1.2mg/kg at 50 days. In the animals that received a subcutaneous injection of benzathine benzylpenicillin alone, the residue levels at the injection sites were 7.8mg/kg at 14 days after treatment. In all treatment groups, the residue levels in liver were <0.05mg/kg at 14 days after dosing. These findings demonstrated that the risk of residues persisting at injection sites increases when high doses of long-acting formulations are administered.
A study was conducted using groups of six pigs, which were fed a combination of sulfadimidine (330mg/kg in the diet), chlortetracycline (330mg/kg in the diet) and procaine benzylpenicillin (160mg/kg in the diet) and killed 0, 2, 4 and 8 days after treatment. Benzylpenicillin residues were detected only in kidney (0.02mg/kg) from one of the pigs in the group killed immediately after treatment; none of the other samples contained any detectable benzylpenicillin residues. The tissues were analysed by an HPLC method with a limit of detection of 0.005mg/kg for muscle, liver and kidney.

In another study in pigs, each pig received three successive daily intramuscular injections of procaine benzylpenicillin at 15000IU/kg of body weight. The pigs were killed in groups 1, 2, 3, 4, 5 and 8 days after the final injection. The residue levels were determined using a liquid chromatography method with a limit of detection of 0.005mg/kg for all tissues. The highest residue levels were found in kidney, where they ranged from 1.3mg/kg at day 1 to 0.24mg/kg at day 3 and <0.005mg/kg at day 8. The only other tissue in which the residue levels exceeded 0.05mg/kg was skin at day 1 (0.08mg/kg). Liver samples were not analysed for residues in this study. The residue levels in the injection-site tissue were below the MRL at 5 days after the final injection.

One-day-old broiler chicks were treated with procaine benzylpenicillin at 28mg/kg in the diet for 42 days. No residues of procaine benzylpenicillin were detectable in any of the tissues (kidney, liver and muscle) tested. The tissues were analysed using a thin-layer chromatography (TLC)–bioautography method with a limit of detection of 0.01mg/kg. The concentrations of benzylpenicillin used in this experiment were significantly higher than the recommended dose of 2.2mg/kg in the diet.

Analytical methods
At its thirty-sixth meeting, the Committee noted the availability of bioassay methods for measuring benzylpenicillin residues in milk with detection limits between 0.001 and 0.010mg/l. Such methods were also available for the analysis of residues in tissues at the concentrations of interest, but the Committee also observed that these methods were not specific for benzylpenicillin and required confirmation by HPLC or mass spectrometry (MS). The available chemical methods had detection limits of 0.05–0.10mg/kg for tissues and 0.01–0.05mg/l for milk and were therefore not sufficiently sensitive for regulatory purposes.
At its present meeting, the Committee noted that for HPLC, MS and other physicochemical methods for residue analysis, the target analyte is typically benzylpenicillin. These methods do not usually distinguish between the various formulations, which include procaine benzylpenicillin, benzathine benzylpenicillin and sodium and potassium benzylpenicillin. It was further noted that several comprehensive reviews have been published since 1990 that provide an excellent picture of the methods available for analysis of residues of benzylpenicillin, and that two of these methods meet the criteria for provisional methods established by the Codex Committee on Residues of Veterinary Drugs in Foods (5). Both of these methods were considered to have demonstrated sufficient analytical sensitivity to meet requirements for regulatory authorities that have adopted the MRLs recommended at the thirty-sixth meeting. The Expert Committee noted that a number of analytical methods may now be found in the scientific literature, using various techniques, including HPLC and gel electrophoresis, and that some of these methods have limits of detection of 0.002 mg/l for milk and 0.005 mg/kg or less for edible tissues. Some such methods may be suitable for further consideration by the Codex Committee. The Expert Committee also noted that there have been significant improvements in the sensitivity of mass spectrometry techniques, making confirmation of the presence of residues at the recommended MRLs of 0.004 mg/l for milk and 0.05 mg/kg for muscle, liver and kidney feasible with current equipment. A variety of rapid test techniques using bioassays or the enzyme-linked immunosorbent assay (ELISA) are also available for screening purposes.

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

National regulatory authorities should note that different formulations and modes of administration, as well as the use of doses outside the recommended dose range, may result in more persistent residues in excess of the MRLs, particularly in organ tissues and at injection sites when slow-release formulations are used. Suitable analytical methods are available for application as screening, quantitative or confirmatory tests in a regulatory monitoring programme.
Maximum Residue Limits
The Committee considered that the MRLs for benzylpenicillin established at its thirty-sixth meeting remain appropriate and are applicable to residues resulting from the use of procaine benzylpenicillin. The MRLs are 50µg/kg for muscle, liver and kidney (all species) and 4µg/l for milk. On the basis of the available data, the recommended tissues for regulatory monitoring are kidney or liver, while muscle is considered to be the target tissue for testing for international trade purposes.

3.2.3 Sarafloxacin
Sarafloxacin is a fluoroquinolone antibacterial agent that acts by inhibiting the activity of DNA gyrase. It is used for the treatment and control of bacterial infections in broiler chickens and turkeys caused by *Escherichia coli* and *Salmonella* spp. Sarafloxacin had not been previously evaluated by the Committee.

Toxicological data
The Committee considered data from a range of toxicological studies on sarafloxacin, including studies on pharmacodynamics, pharmacokinetics, metabolism, acute and short-term toxicity, carcinogenicity, genotoxicity, reproductive toxicity and teratogenicity, special studies on microbiological effects and ecotoxicity and observations in humans. All studies were conducted according to appropriate standards for study protocol and conduct.

The absorption, metabolism and excretion of sarafloxacin were studied in mice, rats, rabbits, dogs and humans. Following oral administration, absorption ranged from a minimum of 10% in humans given a single dose of 800mg to a maximum of 70% in dogs given a single dose of 10mg/kg of body weight. In mice, rats and rabbits, the primary route of excretion was in the faeces. After oral administration of radiolabelled sarafloxacin (10mg/kg of body weight) to mice, rats, rabbits and dogs, the parent drug accounted for approximately 80 to 90% of the total radiolabelled drug recovered from the urine and faeces. This suggests that sarafloxacin undergoes little metabolism in these species. In humans given single oral doses ranging from 100 to 800mg, sarafloxacin accounted for 75 to 80% of the total residues in urine and a metabolite, 3'-oxo-sarafloxacin, comprised approximately 15% of the total residues. In all species studied, a decrease in the fraction of the dose absorbed was seen at high doses.

Orally administered sarafloxacin was found to be slightly hazardous in acute toxicity studies in mice and rats, with LD_{50} values of the order of >5000 to >8000mg/kg of body weight.
In a 90-day oral toxicity study with a 1-month interim kill, rats were
dosed with sarafloxacin at 0, 20, 75, 280 or 1000 mg/kg of body weight
per day by gavage. The only treatment-related effect observed in the
animals killed after 1 month was gross enlargement of the caecum in
animals in the groups dosed at 75 mg/kg of body weight per day and
above. This effect was also found in males treated at 75 mg/kg of body
weight per day and above for 90 days. No microscopic pathological
changes were detected in the enlarged caeca. At necropsy, swollen
ears were reported in two of the animals (about 15 of each sex) given
20 mg/kg of body weight per day for 90 days, and in one, one and
three, respectively, of those dosed at 75, 280 and 1000 mg/kg of body
weight per day. The Committee concluded that this finding was of no
toxicological significance. In the highest-dose group, auricular chon-
dritis was observed histologically in three females. A total of three
deaths occurred in this study in the highest-dose group only. One of
these deaths may have been treatment-related; however, due to the
presence of autolysis in several tissues from this animal, a definitive
cause of death could not be determined. The NOEL was 20 mg/kg of
body weight per day based on grossly enlarged caeca observed at
doses of 75 mg/kg of body weight per day and above.

The potential for sarafloxacin to induce arthropathy in dogs was
evaluated in two 2-week pilot studies. Arthropathy, characterized by
flattening of the angle of the radiocarpal joint, with no microscopic
evidence of articular lesions was observed in young adult (age not
stated) dogs given sarafloxacin at 800 mg/kg of body weight per day by
gelatine capsule. Arthropathy was also observed in 3-month-old dogs
given 125 or 300 mg/kg of body weight per day by gelatine capsule for
2 weeks. Vesicular arthropathic changes of the articular cartilage,
moderate to severe in nature, were observed microscopically in dogs
that received 300 mg/kg of body weight per day. The NOEL was
50 mg/kg of body weight per day for the arthropathic effect of
sarafloxacin in young dogs.

A 90-day oral toxicity study with a 1-month interim kill was con-
ducted in dogs aged between 9 and 14 months. Three groups of
seven male and seven female dogs received sarafloxacin at 0, 5, 25 or
125 mg/kg of body weight per day by gelatine capsule. Decreases in
mean serum globulin were observed in males and females in all
dose groups after 28 days of treatment. After 90 days of treatment,
statistically significant decreases were apparent in females dosed
at 25 and 125 mg/kg of body weight per day and in males in the
highest-dose group. A dose-related decrease in body-weight gain was
observed in males. The NOEL was 5 mg/kg of body weight per day,
based on the decreases in mean serum globulin levels observed at higher doses.

A 90-day oral toxicity study was conducted in 4-month-old dogs. Sarafloxacain was administered by gelatine capsule to two groups of four males and four females at a dose of 10 or 50mg/kg of body weight per day. A third group consisting of six males and six females received 200mg/kg of body weight per day. During the first 2 weeks of the study, sarafloxacain (as the hydrochloride salt) was administered on an actual weight basis without regard to its free base concentration. This resulted in actual dosages that were approximately 80% of the intended free base dosages (i.e. 8, 40 and 160mg/kg of body weight per day). For the remainder of the study, the dosages were adjusted to the target dose levels. However, the Committee considered that the lower values corresponded to actual intake during the study. After 90 days of treatment, a significant decrease in mean serum globulin was observed in females dosed at 40 and 160mg/kg of body weight per day. Mean serum globulin for females dosed at 8mg/kg of body weight per day was comparable to the value in controls. Treatment-related toxicity included erythema of the earflaps and muzzle in males and females dosed at 40 and 160mg/kg of body weight per day. Generalized erythema was observed in one male in the highest-dose group. Swelling around the eyes, eyelids and earflaps was also seen in two males and two females in the highest-dose group. The NOEL was 8mg/kg of body weight per day, based on decreases in serum globulin and facial swelling and erythema observed in males and females dosed at 40 and 160mg/kg of body weight per day.

A carcinogenicity study was conducted in mice (60 males and 60 females per group) in which sarafloxacain was administered at 0, 1000, 5000 or 20000mg/kg in the diet. This study was terminated after 78 weeks due to high mortality in the mice that received 5000 and 20000mg/kg of the drug. Treatment-related toxic effects included nephrotoxicity in females that were dosed at 5000 and 20000mg/kg and gall-bladder calculi and urolithiasis in males in the highest-dose group. Caecal dilatation was observed in males and females in all treatment groups. Caecal torsion was also observed in males and females dosed at 5000 and 20000mg/kg. No treatment-related toxicity was observed in the lowest-dose group. There was no evidence of carcinogenicity in this study.

A combined long-term toxicity/carcinogenicity study was conducted in rats. Sarafloxacain was given at doses of 0, 1000, 10000 or 25000mg/kg in the diet. The toxicity phase (52 weeks) consisted of 20 males and 20 females per group and the carcinogenicity phase (104
weeks) consisted of 65 males and 65 females per group. For the toxicity phase, the intake of sarafloxacin was equal to 61, 670 or 1700mg/kg of body weight per day. A treatment-related decrease in mean body-weight gain was observed in males and females in the highest-dose group. In animals treated with the highest dose increases in blood urea nitrogen and creatinine levels were observed in females and males at weeks 51 and 52, respectively. Serum levels of total protein were decreased compared to controls for males at all dose levels at every sampling period. These decreased total protein levels were characterized by a significant decrease in serum globulin with a relatively unchanged serum albumin level. A statistically significant decrease in serum levels of total protein and globulin was observed in females dosed at 10000 and 25000 mg/kg in the diet at week 51 only. Absolute and relative kidney weights were significantly increased in females in the highest-dose group. Tubular nephropathy was noted in 10 males and females in the highest-dose group and in one female in the lowest-dose group. Dilatation of the caecum was observed in most of the rats treated with 10000 or 25000mg/kg in the diet, but no histopathological changes were observed in grossly dilated caecae. For the carcinogenicity phase, the intake of sarafloxacin was equal to 54, 580 or 1500mg/kg of body weight per day. Non-neoplastic toxicity was similar to that found in the long-term phase of the study. There was no evidence of a carcinogenic effect.

In genotoxicity studies sarafloxacin produced positive results in vitro in a test for unscheduled DNA synthesis in rat primary hepatocytes, and in a mutation assay and an assay for cytogenetic effects in Chinese hamster ovary cells. The results were negative in an in vitro and an in vivo assay for unscheduled DNA synthesis in rat primary hepatocytes and in an in vivo test for micronucleus formation in mouse bone marrow. On the basis of the results of these assays, the Committee concluded that sarafloxacin is genotoxic in vitro but not in vivo.

A three-generation study of reproductive toxicity was carried out in which rats were given sarafloxacin at doses of 0, 75, 275 or 1000mg/kg of body weight per day by gavage, beginning a minimum of 70 days prior to breeding. Each generation comprised 30 males and 30 females per group. The relative liver weights were significantly decreased in males and females of the second parental generation and in males of the third parental generation in the groups dosed at 275 and 1000mg/kg of body weight per day. Females of the first parental generation in the highest-dose group also had decreased relative liver weights. No treatment-related effects were observed on reproductive parameters, litter parameters or fetal morphology at any of the doses.
tested. The NOEL for parental toxicity was 75 mg/kg of body weight per day, based on decreased liver weights observed in males and females at higher doses.

In a study of developmental toxicity, sarafoxacin was administered daily to pregnant rats during days 6–15 of gestation. Four groups of 20 pregnant rats received oral doses by gavage of 0, 20, 75, 280 or 1000 mg of sarafoxacin base/kg of body weight per day. There was no evidence of maternal toxicity or teratogenicity at any of these doses.

A developmental toxicity study was conducted in rabbits given sarafoxacin at doses of 0, 15, 35 or 75 mg/kg of body weight per day by gavage during days 6–18 of gestation. Due to excessive maternal toxicity manifested by decreased body weight, abortions and decreased defecation and urination, the highest dose was considered to be inappropriate for the evaluation of teratogenicity in the rabbit. Maternal toxicity was also observed at doses of 15 and 35 mg/kg of body weight. External, visceral and skeletal malformations were observed in fetuses in the groups dosed at 35 and 75 mg/kg of body weight. Fetotoxicity was also observed at these doses. The NOEL for teratogenicity and fetotoxicity was 15 mg/kg of body weight per day. A NOEL for maternal toxicity was not identified. The teratogenic effects in this study were considered to be secondary to maternal toxicity and not directly attributable to treatment with sarafoxacin.

Data in humans reviewed by the Committee consisted of reports of side-effects in subjects enrolled in clinical trials that compared the safety of oral doses of sarafoxacin to placebos in healthy male volunteers. Dosages ranged from 100 to 800 mg/person per day for 1–7 consecutive days. The effects on gastrointestinal microflora were not evaluated in these studies. The side-effects reported included asthenia, vasodilatation, anxiety, dizziness, and nervousness or somnolence, which were observed sporadically at all dose levels.

**Microbiological data**

Sarafoxacin belongs to a group of antimicrobial fluoroquinolones that are primarily active against aerobic Gram-negative bacteria. In humans, this characteristic is used therapeutically for the selective elimination of potential aerobic and facultative anaerobic pathogens from the gastrointestinal tract while preserving the predominant anaerobic bacterial intestinal flora that protect the gastrointestinal tract from invasion or overgrowth by potentially pathogenic bacteria.

Several *in vitro* studies on the antimicrobial activity of sarafoxacin were evaluated by the Committee. In one study MIC$_{50}$ and MIC$_{90}$ (the minimum sarafoxacin concentration resulting in 90% inhibition)
values were determined from a total of 735 human clinical isolates from 65 different genera. The inoculum density in this study was $10^4$ bacteria per inoculation. The study assessed the effect of sarafloxacin against 210 bacterial strains, 14 of which were identified as possible constituents of the human intestinal microflora. *Escherichia coli* and *Enterobacter cloacae* were the most sensitive organisms with MIC$_{50}$ values of less than 0.031 µg/ml. *Peptostreptococcus* was the most sensitive relevant organism, with an MIC$_{50}$ value of 0.125 µg/ml. *Bacteroides vulgatus* was the least sensitive relevant organism, with an MIC$_{50}$ value of 8 µg/ml.

In another study designed to evaluate the effect of inoculum size on the potency of sarafloxacin in vitro, the geometric mean MIC value was calculated for sarafloxacin against 50 clinical isolates of human origin. *Escherichia coli* was the most sensitive organism tested, the geometric mean MIC values being 0.018 µg/ml at an inoculum density of $10^5$ colony-forming units per ml and 0.05 µg/ml at an inoculum density of $10^7$ colony-forming units per ml.

The antimicrobial activities of four potential metabolites of sarafloxacin were determined in MIC assays. The MIC$_{50}$ values of the metabolites varied depending on the species tested, but in general they were significantly higher than those for sarafloxacin against *E. coli*. Therefore, the Committee concluded that the antimicrobial activity of the metabolites against relevant strains of bacteria found in the human gastrointestinal tract would be significantly lower than that of sarafloxacin.

The frequency of occurrence of spontaneous resistance of human clinical isolates to sarafloxacin was studied using *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* cultured on agar plates containing sarafloxacin at four and eight times the MIC$_{50}$, and by repeatedly transferring organisms into broth containing increasing concentrations of sarafloxacin. The results showed that the resistant mutants were stable.

The effects of sarafloxacin against five strains each of *E. coli*, *Bacteroides fragilis* and *Bifidobacterium* spp. of human origin were assessed in an *in vitro* model of the gastrointestinal system. The model was designed to simulate the possible inactivation of sarafloxacin due to degradation and binding of the drug in food. *E. coli* and *Bacteroides fragilis* strains grew in the presence of higher concentrations of sarafloxacin in the model than in broth culture. The results indicated that sarafloxacin binds to organic matter in the gastrointestinal model and decreases the sensitivity of the *E. coli* strains to the compound as compared to broth culture. The results of this study suggest that
sarafloxacin was less available in the gastrointestinal model than in broth culture.

The effect of pH on the potency of sarafloxacin in vitro was studied using aerobic and anaerobic clinical bacterial isolates of human origin. In general, for those organisms considered to be potential constituents of the human gastrointestinal tract, the geometric mean MIC value increased as pH decreased.

The Committee noted that the organisms most sensitive to the antimicrobial effects of sarafloxacin, namely E. coli and Enterobacter cloacae, comprise approximately 1% of the total bacterial population of the gastrointestinal tract. They are considered to be of minimal importance in the natural resistance of the gastrointestinal tract to colonization by pathogenic bacteria. The available microbiological data did not permit a full evaluation of the relevant bacteria of the human gastrointestinal tract because very few strains of relevant bacteria had been tested. However, on the basis of a published report on the activity of related fluoroquinolones against relevant human intestinal anaerobic bacteria it was shown that Clostridium perfringens and Peptostreptococcus spp. were the most sensitive strains tested. In tests on relevant bacteria the lowest MIC₅₀ (0.125 µg/ml) for sarafloxacin was also seen in Peptostreptococcus spp. Only three strains rather than the preferred 10 strains were tested, but the Committee considered that these data were sufficient to support an ADI.

In calculating an ADI based on antimicrobial activity, the Committee used the formula described in section 3.2:

\[
\text{Upper limit of ADI} = \frac{0.125 \text{µg/g} \times 220 \text{g}}{0.70 \times 2^2 \times 60 \text{kg}} = 0.33 \text{µg/kg of body weight}
\]

The Committee established an ADI of 0–0.3 µg/kg of body weight for sarafloxacin, based on its antimicrobial activity against Peptostreptococcus, which was the most sensitive relevant organism isolated from the human gastrointestinal flora for which limited but

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4 For the purpose of this evaluation, the MIC₅₀ value is the MIC₅₀ for sarafloxacin against three strains of human clinical isolates of Peptostreptococcus, which was the most sensitive relevant organism of the human gastrointestinal microflora tested with sarafloxacin.

5 The mass of the colonic contents.

6 The fraction of the dose available to the gastrointestinal microflora was determined to be approximately 70%, based on studies in humans given an oral dose of 100 mg of sarafloxacin, in which about 30% was absorbed.

6 A safety factor of 2 was used because of the limited MIC data available on the sensitive relevant bacteria of the human gastrointestinal tract.

* The weight of an adult person.
adequate microbiological data were available. The Committee noted that this ADI provided a margin of safety of 17,000 when compared to the lowest toxicological NOEL of 5mg/kg of body weight per day in the 90-day study in dogs.

**Metabolism data**
Sarafloxacin is readily absorbed and rapidly excreted by rats, mice, dogs, rabbits, chickens and turkeys. When chickens and turkeys received four daily doses of $[^{14}C]$sarafloxacin hydrochloride by gavage on 5 successive days, more than 80% of the dose was excreted in the first 6 hours after treatment.

The metabolism of $[^{14}C]$sarafloxacin was studied in chickens and turkeys. No differences in metabolism were observed between males and females, but the metabolic profiles in the liver differed between the two species. The main route of metabolism in poultry liver is the formation of a sulfamic acid conjugate at the N-position in the piperazine ring and/or a glucuronide with the carboxy group. The unidentified minor metabolites were also conjugates because acid or base hydrolysis of the metabolites yielded parent sarafloxacin. More conjugates were present in turkey liver (47–57%) than in chicken liver (8–13%). The parent drug accounted for 21% of the total residues in turkey liver and 67% of the total residues in chicken liver.

**Residue data**
All the studies examined the depletion of residues in equal numbers of male and female birds. The analysis of the results indicated that there were no significant differences between the values for males and females; therefore the results for both sexes were combined.

**Chickens (broilers).** Three-week-old chickens (mean body weight 623 g) were given $[^{14}C]$sarafloxacin hydrochloride at a dose of 0.50mg (equivalent to 0.8mg/kg of body weight) by gavage four times daily for 5 days. Groups of six birds were killed 6, 18, 36 and 72 hours after drug withdrawal. Samples of light muscle, dark muscle, liver, fat and skin with adhering fat were collected and the concentrations of the total radioactive residues (as sarafloxacin equivalents) were measured by sample combustion and/or scintillation counting. At 6 hours after dosing, the residue levels in sarafloxacin equivalents were: 35µg/kg (range 24–45µg/kg) in light muscle, 28µg/kg (18–38µg/kg) in dark muscle, 320µg/kg (221–482µg/kg) in liver, 22µg/kg (8–65µg/kg) in fat and 29µg/kg (19–39µg/kg) in skin with fat. The residue levels were below the limit of detection (22µg/kg) in muscle and fat at later sampling times. In skin with fat, the residue levels were 26µg/kg (range <4–48µg/kg) at 18 hours and below the limit of detection.
(21 µg/kg) at 36 hours. In liver the residue levels were 70 µg/kg (range 21–219 µg/kg) at 18 hours, 21 µg/kg (17–28 µg/kg) at 36 hours and below the limit of detection (15 µg/kg) at 72 hours. Thus, the residue levels were highest and most persistent in liver. At 24 hours after withdrawal of the drug, residues were measurable only in liver and skin. No residues were detected in any of the tissues at 72 hours after drug withdrawal.

Broilers weighing 1.8–2.5 kg were given sarafloxacin in their drinking-water at a concentration of 15.5–18.0 mg/l (equivalent to 2.7 mg/kg of body weight per day) for 119 hours. Groups of six birds were killed 0, 26, 96 and 122 hours after drug withdrawal. Samples of muscle, liver, fat, kidney and skin were collected and the concentrations of sarafloxacin residues measured by HPLC. The mean levels of residues measured as parent drug were highest in liver: 480 µg/kg (range 191–929 µg/kg) at 0 hours, 6 µg/kg (5–7.5 µg/kg) at 26 hours and below the limit of detection (2.5 µg/kg) at 96 hours. Residues were detected at all sampling times in skin with adhering fat; the mean concentrations were 44 µg/kg at 0 hours, 19 µg/kg at 26 hours, 8 µg/kg at 96 hours and 9 µg/kg at 122 hours. No residues were found in fat, and in muscle and kidney, residues were found only at 0 hours, when the mean concentrations were 36 µg/kg (21–62 µg/kg) and 230 µg/kg (112–550 µg/kg), respectively.

Turkeys. Turkeys weighing about 2.7–3.7 kg received [14C]sarafloxacin hydrochloride at a dose of 4.25 mg (equivalent to approximately 1.75 mg/kg of body weight) by gavage four times daily for 5 days. The total dose was 21 mg per bird (equivalent to approximately 7 mg/kg of body weight) per day, which was higher than the recommended field dose of 4 mg/kg of body weight per day (30 mg/l in drinking-water). Groups of six birds were slaughtered 6, 18, 36 and 72 hours after drug withdrawal. Samples of light muscle, dark muscle, liver, fat and skin with adhering fat were collected and the concentrations of the total radioactive residues (as sarafloxacin equivalents) were measured by sample combustion and/or scintillation counting. At 6 hours after treatment the mean residue concentrations in sarafloxacin equivalents were: 12 µg/kg in light muscle, 12 µg/kg in dark muscle, 390 µg/kg in liver, 52 µg/kg in fat and 28 µg/kg in skin with fat. The residues were below the limit of detection (13 µg/kg) in muscle at 18 hours and at later sampling times. The mean residue concentrations in fat were 27 µg/kg at 18 hours and below the limit of detection (25 µg/kg) at 36 hours. In skin with adhering fat the mean residue concentrations were 22 µg/kg at 18 hours, 19 µg/kg at 36 hours and 20 µg/kg at 72 hours. In liver the mean residue concentrations were 87 µg/kg at 18 hours, 60 µg/kg at 36 hours and 35 µg/kg at 72 hours.
In another study, turkeys weighing 6.0–8.7 kg were given sarafloxacin in drinking-water at a concentration of 21.1–28.5 mg/l (equivalent to 2.9 mg/kg of body weight per day) for 5 days. Groups of six birds were killed at 0, 24 and 120 hours after drug withdrawal. Samples of muscle, liver, kidney, fat and skin were collected and the concentrations of sarafloxacin measured by HPLC. The mean residue concentrations in muscle were 5 μg/kg at 0 hours and below the limit of detection (2.5 μg/kg) at later sampling times. No residues were detected in fat at any time. The mean residue concentrations of sarafloxacin were highest in skin: 44 μg/kg at 0 hours, 19 μg/kg at 24 hours and 8 μg/kg at 120 hours. In liver the mean residue concentrations were 34 μg/kg at 0 hours, 5 μg/kg at 26 hours and below the limit of detection at later sampling times. In kidney the mean residue concentration was 12 μg/kg at 0 hours and below the limit of detection at later sampling times. The residues of sarafloxacin persisted in skin as observed in the residue-depletion study using radiolabelled drug. The residue concentrations in both skin and muscle suggest that the parent drug is the major residue in these tissues.

*Choice of marker residue.* The parent drug is the clear choice as marker residue because it is the major residue in all tissues. It is neither necessary nor possible to correlate the values observed for the total residues with those found for the parent drug in the residue-depletion study using unlabelled drug, because the sponsors have shown that the antimicrobial activities of some of the metabolites (N-acetylsarafloxacin, N-formylsarafloxacin, 3'-oxo-sarafloxacin and the sulfamic acid conjugate of sarafloxacin) are significantly lower than that of sarafloxacin.

*Choice of target tissues.* The levels of residues in poultry are highest in liver and kidney and persist in skin with adhering fat. The kidney is not normally a target tissue for poultry and therefore the main target tissues should be liver and skin with adhering fat (skin and fat). Because muscle is the major edible tissue of poultry and residues are found in this tissue shortly after drug withdrawal an MRL was recommended for muscle.

*Bioavailability*  
The bound residues were extracted from samples of chicken and turkey liver with acidic and basic acetonitrile; 13–18% of the residues could not be extracted. Neither the identity nor the antimicrobial activity of the bound residues was investigated.

*Analytical methods*  
The analytical methods specifically measure free sarafloxacin but not sarafloxacin conjugates which form the majority of the total residues
in turkey liver (47–57%), but account for only 8–13% of the total residues in chicken liver. The methods consist of extracting homogenized tissue (muscle, liver, kidney and skin with adhering fat) with an organic solvent, drying, dissolving the extract in the mobile phase, and analysis using HPLC with fluorescence detection. The characteristics of the method used by the sponsor are detailed for both broilers and turkeys. The method was linear over the concentration range of 5–4000μg/kg and the limit of quantification for all poultry tissues was claimed to be 5μg/kg. The recoveries for poultry muscle, liver, kidney and skin with adhering fat ranged between 57% and 67%. The recoveries from fat were 93% for broilers and 100% for turkeys. The method has not been tested in a collaborative study with other laboratories, but good reproducibility was achieved in the sponsor’s laboratory when three different persons used four different HPLC columns of the same type. There was no interference in the chromatograms from monensin, narasin, salinomycin, flumequine, enrofloxacin, difloxacin or danofloxacin. Interference from the tissue matrix was less than 5μg/kg. The method was suitable for the routine analysis of a large number of samples per day.

Maximum Residue Limits
In reaching its decision on MRLs for sarafloxacin, the Committee took the following factors into account:

- The ADI is 0–0.3μg/kg of body weight. This corresponds to a maximum ADI of 18μg for a 60-kg person.
- The parent drug is the marker residue.
- Only the parent drug has significant antimicrobial activity.
- The quantification limit of the analytical method is 5μg/kg.
- As no residues are detectable in poultry muscle at 18 hours after withdrawal, the MRL should be set at twice the limit of quantification of the analytical method.
- The main target tissues are liver and skin with adhering fat.
- The MRLs apply equally to chickens and turkeys.

The Committee recommended MRLs for sarafloxacin of 10μg/kg for muscle, 80μg/kg for liver, 80μg/kg for kidney and 20μg/kg for fat/skin, expressed as parent drug, in chickens and turkeys. From these values for the MRLs, the maximum theoretical daily intake would be 16μg.

3.2.4 Spectinomycin
Spectinomycin was previously evaluated at the forty-second meeting of the Committee, when an ADI of 0–40μg/kg of body weight was established, based on a microbiological end-point (Annex 1,
reference 110). Temporary MRLs were recommended for muscle, liver, kidney and fat in cattle, pigs and chickens, as well as for cows’ milk. The MRLs were designated as temporary because many of the residue-depletion data were from either interim progress reports or pilot studies.

At the present meeting, the Committee reviewed new information that had been submitted by the two sponsors, including the results of pharmacokinetic studies in cattle, pigs and sheep, data from residue-depletion studies in cattle, pigs, sheep and chickens, studies in support of the recommended MRLs for sheep, and studies in support of new MRLs for muscle and fat. Information was provided on two analytical methods for the quantification of residues, including validation data on one of the methods, and a method for confirming the presence of residues. A study comparing the microbiological inhibition and chemical assay methods was also provided.

**Pharmacokinetic data**

In pharmacokinetic studies, cattle and sheep given an injectable solution of radiolabelled spectinomycin at a dose of 10mg/kg of body weight showed nearly identical pharmacokinetic parameters. The drug was rapidly absorbed and all of the dose was shown to be bioavailable. There was little difference between intramuscular and subcutaneous treatments. In a study in cattle given five successive daily subcutaneous injections of radiolabelled spectinomycin at a dose of 15mg/kg of body weight, more than 90% of the radioactivity was eliminated within 24 hours after the last dose. The residues were eliminated from plasma with a half-life of about 8 days. Most (69–84%) of the dose was eliminated in the urine, with lesser amounts being eliminated in the faeces and tissues (5–8% and 1–3%, respectively). The residue levels in tissues 1–15 days after treatment were highest in kidney and liver (85–90% of the residues), with muscle and fat containing much lower amounts.

In a study in 16 calves given radiolabelled spectinomycin, eight metabolites were identified in the urine by HPLC/MS. The parent drug accounted for 62–64% of the total residues in urine, while the other metabolites accounted for less than 9% each. The major residue identified in kidney was spectinomycin, and the major residue in liver was dihydrospectinomycin. The parent drug accounted for 6.6–15% of the total residues in kidney and for less than 4.2% of the total residues in liver. The concentrations of residues in muscle and fat were too low to allow the profile and identity of the metabolites to be determined. Since the ADI is based on a microbiological end-point, only the antimicrobial activity of the metabolites needs to be known.
These studies supported those reviewed at the forty-second meeting of the Committee, identifying the kidney as the target tissue and spectinomycin as the marker residue.

Pharmacokinetic studies were conducted in pigs given an intramuscular injection of spectinomycin free base equivalents at a dose of 15 mg/kg of body weight as either the hydrochloride or the sulfate salt. The results were comparable to those in cattle, but somewhat higher values for the AUC and shorter times to maximum concentrations in plasma indicated a more rapid absorption in pigs.

A study in pigs that received radiolabelled spectinomycin at a dose of 88 mg/kg of medicated feed (1:1 ratio of lincomycin and spectinomycin) (equivalent to approximately 2.7 mg/kg of body weight per day) for 7 days was reported. The main route of excretion of radioactivity was in the faeces (72%), followed by the urine (7.2%). In kidney, the concentrations of total residues were 0.46 mg/kg at 1 day after drug withdrawal and had declined to 0.02 mg/kg by day 10. In liver, the concentrations of total residues were 0.21 mg/kg at day 1 and had declined to 0.02 mg/kg by day 10. No residues were detectable in muscle tissue after correcting for tritiated water. The levels of residues in fat remained constant between days 1 and 10 at about 0.15 mg/kg. The concentrations of total residues in all tissues were low; this is consistent with the poor availability of the drug following oral administration.

The pharmacokinetic data in sheep were obtained in a study that was identical to that carried out in cattle. The pharmacokinetic parameters were nearly identical to those in cattle after a subcutaneous or intramuscular injection of spectinomycin at a dose of 15 mg/kg of body weight. Spectinomycin was bioavailable and after administration of three successive daily doses, there was no significant difference between sheep and cattle in the pharmacokinetic parameters measured, and also no indication of accumulation of residues from day 1 to day 3.

Residue data
Three residue-depletion studies in cattle were reported, one using radiolabelled spectinomycin and two with the unlabelled drug. In the study using radiolabelled drug, cattle received a subcutaneous dose of 15 mg/kg of body weight and residue levels were determined at 1, 5, 10 and 15 days after treatment. The levels of total residues in each tissue were determined by measuring the radioactivity, and the levels of spectinomycin residues were determined by HPLC (limit of quantification 0.1 mg/kg). The levels of total residues in kidney declined from
60 mg/kg on day 1 to 2.7 mg/kg on day 15. The levels of spectinomycin residues in kidney were 9.1 mg/kg on day 1 and 0.20 mg/kg on day 15. The levels of total residues in liver were 33 mg/kg on day 1 and had declined to 4.5 mg/kg by day 15. The levels of spectinomycin residues were 1.4 mg/kg on day 1 and 0.14 mg/kg on day 15. Only levels of total residues could be determined in muscle and fat. In muscle, the levels of total residues were 1.0 mg/kg on day 1 and 0.29 mg/kg on day 15. The corresponding levels in fat were 1.3 mg/kg on day 1 and 0.77 mg/kg on day 15.

In the first residue-depletion study using unlabelled spectinomycin, 24 beef cattle received five consecutive daily doses of 15 mg/kg of body weight. Similar amounts of spectinomycin residues were measured in muscle, liver, kidney and fat. In the second study, calves received five successive daily injections of unlabelled spectinomycin at a dose of 30 mg/kg of body weight. The levels of residues were higher than in the first study. On day 1 after withdrawal of the drug, the level of spectinomycin residues was 110 mg/kg in kidney, declining to 2.8 mg/kg on day 14. In liver, the levels of residues on day 1 and day 14 were 6.4 mg/kg and 0.90 mg/kg, respectively. For muscle, the levels of residues on day 1 and day 14 were 1.2 mg/kg and 0.20 mg/kg, respectively; the residues in fat were below the limit of quantification.

Because the ADI is based on a microbiological end-point, one sponsor reported two studies that evaluated the relationship of the microbiological inhibition assay with their HPLC method in liver and kidney. The microbiological analysis used a cylinder plate assay that was not very sensitive to spectinomycin (limit of quantification 4 mg/kg), while the HPLC assay had a reported limit of quantification of 0.1 mg/kg. For kidney the ratio of the levels of residue measured by the microbiological assay to those measured by HPLC was approximately 0.98; for liver the ratio was approximately 3.6. This result supports the use of the HPLC method for the analysis of residues in kidney tissue. Since the only microbiologically active residue in liver is dihydropectinomycin, this metabolite is responsible for almost all the activity found there, even though it accounts for less than 10% of the antimicrobial activity of the parent drug.

One new study in lactating cows which received three daily injections of spectinomycin at a dose of 10 mg/kg of body weight for 5 days confirmed the results of a study reviewed by the Committee at its forty-second meeting. The levels of residues in milk had depleted to below the limit of quantification (0.10 mg/l) by 48 hours after treatment.

Two residue-depletion studies in pigs given the unlabelled drug were reported. In the first study, 12 pigs received a single intramuscular
injection of spectinomycin hydrochloride or spectinomycin sulfate at a dose of 15 mg/kg of body weight. The level of residues in kidney following treatment with the hydrochloride salt was 9.6 mg/kg on day 1 and had declined to 1.9 mg/kg by day 5. The level of residues at the injection site declined from 4.8 mg/kg on day 1 to 0.8 mg/kg on day 5. Following treatment with the sulfate salt, the corresponding levels of residues in kidney were 11 mg/kg on day 1 and 2.3 mg/kg on day 5. The levels of residues at the injection site were 3.5 mg/kg on day 1 and 0.7 mg/kg on day 5. In the second study, pigs were treated with an oral solution of spectinomycin at a dose of 25–29 mg/kg of body weight twice daily for 5 days. The level of residues in kidney on day 1 was 18 mg/kg and had declined to below the limit of quantification (0.5 mg/kg) by day 14. In liver the level of residues on day 1 was 2.2 mg/kg and had declined to below the limit of quantification (0.5 mg/kg) by day 10. The level of residues in muscle tissue was 0.64 mg/kg on day 1 and below the limit of quantification (0.3 mg/kg) on day 3. For fat, the levels of residues were 0.69 mg/kg on day 1 and below the limit of quantification (0.25 mg/kg) on day 7.

A residue-depletion study in 7–8-week-old broiler chickens was reported. The chickens received an oral solution of 100 mg of spectinomycin and 50 mg of lincomycin/kg of body weight per day in drinking-water for 7 days. The levels of residues in kidney declined from 2.0 mg/kg at the time of withdrawal to <0.1 mg/kg on day 4. The levels of residues in liver declined from 0.43 mg/kg at the time of withdrawal to <0.1 mg/kg on day 4. The levels of residues in muscle were almost identical to those in liver at all sampling times. The levels of residues in skin and adhering fat were 2.9 mg/kg at the time of withdrawal, declining to 0.3 mg/kg on day 8.

In a second residue-depletion study, broiler chickens were treated with spectinomycin at a dose of 50 mg/kg of body weight per day in drinking-water for 5 successive days. No spectinomycin residues could be quantified in any sample on day 1 after withdrawal. The limits of quantification for the analytical method used by the sponsor were 0.3 mg/kg for muscle, 0.5 mg/kg for liver and kidney, and 0.25 mg/kg for fat. These findings are consistent with those obtained in other species of food-producing animals (i.e. relatively low levels of residues following oral administration of spectinomycin).

No new studies on the depletion of spectinomycin residues in eggs were reported; however, such a study was reported at the forty-second meeting of the Committee. In that study, laying birds were treated with a 1:1 mixture of spectinomycin and lincomycin at a dose of 220, 330 or 440 mg/kg of feed or with a 2:1 mixture of
spectinomycin and lincomycin at a dose of 0.5 g/l in the drinking-water for 7 days. No residues were detected in eggs in any group during the last 2 days of treatment or the first 3 days following withdrawal. The residues were assayed using a microbiological method with a limit of quantification of 2 mg/kg.

Two new residue-depletion studies in sheep were reported. In the first study, sheep received three successive daily intramuscular injections of a 2:1 mixture of spectinomycin and lincomycin at a dose of 15 mg/kg of body weight. The levels of residues in kidney declined from 12 mg/kg at 8 hours after withdrawal to 0.1 mg/kg on day 10. In liver, the levels of residues declined from 0.63 mg/kg at 8 hours after withdrawal to 0.08 mg/kg on day 10. The levels of residues in muscle were 0.29 mg/kg at 8 hours after withdrawal and <0.04 mg/kg on day 7. The levels of residues in fat were 0.19 mg/kg at 8 hours after withdrawal and <0.04 mg/kg on day 7. In the second study, sheep received spectinomycin intramuscularly at a dose of 30 mg/kg of body weight twice daily for 5 days. The levels of residues in kidney were 100 mg/kg on day 1 after withdrawal and had declined to 0.78 mg/kg on day 18. The levels of residues in liver were 4.8 mg/kg on day 1 and <0.5 mg/kg on day 18. In muscle, the levels of residues declined from 0.43 mg/kg on day 1 to <0.15 mg/kg on day 7. In fat, the levels of residues were 0.41 mg/kg on day 1 and <0.25 mg/kg on day 3.

Analytical methods
One sponsor reported the results of performance trials on three methods, including two quantitative methods and one confirmatory method for spectinomycin residues. The quantitative method preferred by one sponsor involves solvent extraction and solid-phase extraction followed by an HPLC procedure employing a gradient elution for separation with post-column oxidation and derivatization to allow fluorescence detection. The method showed no potential interference with seven other antibiotics examined. The performance of the method was tested in two laboratories using fortified samples and samples with incurred residues over a concentration range of 0.1–10.0 mg/kg in kidney and 0.1–1.0 mg/kg in muscle, liver, and fat. The performance values for recovery, day-to-day variability and limit of quantification were evaluated; the recoveries in all tissues were greater than 80%. The second quantitative method was not as reliable as the first; however, the results obtained for spectinomycin in bovine kidney by the two methods were within 5.6% of each other when the 95% confidence limits were applied. The confirmatory method was based on mass spectrometry. The Committee concluded that the three methods are satisfactory for monitoring purposes.
The second sponsor also reported the results of tests on the performance of an analytical method for quantification of spectinomycin residues using a similar extraction and isolation procedure, derivatization and HPLC with ultraviolet detection. The performance of the method was tested in only one laboratory. Although no study on optimizing the performance of the method was reported, no interference was noted when the method was used to examine tissues containing spectinomycin residues and residues of three other antimicrobial drugs. The recoveries in all species and tissues were satisfactory for monitoring purposes; however, the limits of quantification were higher than with the HPLC method noted above. The Committee concluded that this method may be suitable for the routine analysis of spectinomycin residues, depending on the analytical equipment available.

Maximum Residue Limits
In reaching its decision on MRLs for spectinomycin, the Committee took into account the following information:

• An ADI of 0–40µg/kg of body weight, based on a microbiological end-point, was established at the forty-second meeting of the Committee. This corresponds to a maximum ADI of 2400µg for a 60-kg person.

• The parent drug is the marker residue.

• Kidney is the target tissue. However, in view of the practical difficulties of collecting kidney tissue from chickens for residue analysis, skin and adhering fat may be the more appropriate target tissue in these animals.

• The only microbiologically active residues are the parent drug and dihydrospectinomycin.

• The parent drug is the only microbiologically active residue in muscle, kidney, fat, milk and eggs.

• Dihydrospectinomycin is the major microbiologically active residue in liver.

• The ratio of the concentration of residues in liver measured by the microbiological assay to that measured by HPLC is approximately 4:1. Therefore, spectinomycin accounts for 25% of the total residues in liver.

• The antimicrobial activity of dihydrospectinomycin is approximately 10% of that of the parent drug.

The Committee recommended MRLs in cattle, sheep, pigs and chickens of 500µg/kg for muscle, 2000µg/kg for liver and fat, and 5000µg/kg for kidney, expressed as parent drug. The Committee also recommended MRLs of 200µg/l for cow’s milk and 2000µg/kg for...
eggs, expressed as parent drug. From these values, the theoretical maximum daily intake of spectinomycin residues is 1800μg (Table 2).

3.2.5 Tetracyclines: chlortetracycline, oxytetracycline and tetracycline

The tetracyclines (chlortetracycline, oxytetracycline and tetracycline) are broad-spectrum antimicrobials that are widely used in human and veterinary medicine to treat a variety of bacterial infections.

These products were first evaluated as a group at the twelfth meeting of the Committee (Annex 1, reference 17), at which time a temporary ADI of 0–0.15 mg/kg of body weight was established. Oxytetracycline was re-evaluated at the thirty-sixth meeting of the Committee (Annex 1, reference 97), when an ADI of 0–3μg/kg of body weight was established, based on a NOEL of 2mg per person per day in a study in human volunteers and a safety factor of 10. Tetracycline and chlortetracycline were re-evaluated at the forty-fifth meeting of the Committee (Annex 1, reference 119). At that meeting, the Committee converted the ADI for oxytetracycline to a group ADI of 0–3μg/kg of body weight for oxytetracycline, tetracycline and chlortetracycline, separately or in combination, on the basis of their similar antimicrobial activity. The Committee noted that this ADI provides an adequate margin of safety when compared with the lowest NOEL for toxicological effects of 100mg/kg of body weight per day for chlortetracycline in dogs.
At the Tenth Session of the Codex Committee on Residues of Veterinary Drugs in Foods (3), the Expert Committee was requested to reconsider its previous evaluation of the tetracyclines.

**Microbiological data**

The original toxicological studies used to establish an ADI for oxytetracycline, tetracycline and chlorotetracycline showed that these drugs have low toxicity. Additional toxicological studies performed with oxytetracycline, tetracycline and chlorotetracycline have also shown that these compounds have low toxicity.

Because the tetracyclines are incompletely absorbed from the gastrointestinal tract, they readily reach high concentrations in the intestine, producing perturbations of the intestinal microflora within 48 hours of daily treatment. Experience with tetracyclines in human medicine indicates that therapeutic levels of tetracyclines can perturb the intestinal microflora by inducing the emergence of resistant strains and altering the metabolic activity of the microflora, its resistance to colonization by pathogenic, opportunistic or resistant microorganisms (barrier effect) and its ecological balance, without any identified deleterious effect.

Studies performed in humans have shown that administration of 1–2 g of tetracyclines per day produces adverse effects on the intestinal microflora. In one study the emergence of resistant strains occurred following administration of 100 mg of tetracycline per day and 10 mg of oxytetracycline per day, but resistant organisms existed before treatment and the resistance was transitory. Another microbiological study in humans showed that administration of 50 mg of tetracycline did not alter the shedding of *E. coli* from the intestine, indicating that the barrier effect was not perturbed at this dose. In the study in human volunteers that was used previously by the Committee to establish the ADI for the tetracyclines, therapeutic doses of oxytetracycline (2 g per day for 7 days) produced perturbations in the balance of the intestinal flora with an increased incidence of resistant Enterobacteriaceae and colonization by yeasts. The dose of 20 mg per day produced a marginal impact on the ecology of the intestinal flora by suppressing some susceptible anaerobes, but there was no evidence of resistant Enterobacteriaceae in the faeces following treatment with the dose of 2 mg per day. At its thirty-sixth meeting, the Committee applied a safety factor of 10 to the NOEL of 2 mg per day from the study in humans to establish an ADI of 0–3 μg/kg of body weight per day.
Since the previous evaluation, additional data from microbiological studies on the effects of tetracyclines on the human gastrointestinal microflora have become available.

The results from an in vitro continuous bacterial culture system (chemostat) in which the dosage was equivalent to 0.025, 0.25 or 2.5 mg/kg of body weight per day were available. They showed that a dose equivalent to 2.5 mg of tetracycline/kg of body weight per day produced an increase in the proportion of resistant strains of *E. coli*. The proportion of resistant strains of *E. coli* produced at this dose level increased from <20% to >50% within 24 hours of exposure, and to >60% after 48 hours of exposure. However, the proportion of resistant strains fell to approximately 35% by day 6 of exposure despite continued treatment. In the control chemostat, which contained no drug, the proportion of resistant strains never exceeded 5%. No effects were seen in the chemostats in which the dose was equivalent to 0.025 or 0.25 mg of tetracycline/kg of body weight per day. None of the other microbiological end-points analysed in this study changed with any of the concentrations of tetracycline tested.

These results agree with the findings in the literature concerning the effects of tetracycline on the human intestinal microflora. Most of the studies report a very low impact of tetracycline on the gastrointestinal flora, with the selection of resistant bacterial strains being the most sensitive end-point. On the basis of the new information on the effect of tetracycline on the human intestinal microflora, the Committee concluded that a safety factor was not needed. This was because the selection of resistant Enterobacteriaceae is a very sensitive end-point for evaluating the microbiological effect of tetracyclines on the human intestinal microflora and there is little variation between individuals with respect to this effect. On the basis of the NOEL of 2 mg per day (equivalent to 33 µg/kg of body weight per day) obtained in the study in humans, the Committee established an ADI of 0-30 µg/kg of body weight for the tetracyclines, alone or in combination. The ADI was rounded to one significant figure, in accordance with standard practice.

*Residue data*

Chlortetracycline, oxytetracycline and/or tetracycline were previously evaluated at the twelfth, thirty-sixth, forty-fifth and forty-seventh meetings of the Committee (Annex 1, references 17, 91, 119 and 125). At its forty-seventh meeting, the Committee recommended the following MRLs: 100 µg/kg for muscle, 300 µg/kg for liver and 600 µg/kg for kidney in cattle, pigs, sheep and poultry; 100 µg/l for milk in cattle and sheep; and 200 µg/kg for eggs (poultry). An MRL of
100μg/kg for oxytetracycline in muscle of giant tiger prawns was also recommended. At that meeting, the Committee also evaluated the analytical methods for measuring residues at these MRLs.

At the forty-fifth and forty-seventh meetings of the Committee, the limiting factor in recommending MRLs for the tetracyclines was the low value of the ADI (0–3μg/kg of body weight). As a result some of the values for the MRLs were close to the limit of quantification of the analytical methods available. However, this practical approach based on the limitations of the analytical methods resulted in theoretical maximum daily intake values 30% higher than the ADI.

With the establishment of an ADI of 0–30μg/kg of body weight, the Committee recognized that the constraints placed on the recommended MRLs no longer exist. In particular, the limits of quantification of the currently available methods for determination of residues in tissues that have been performance tested would permit the satisfactory control of residues at twice the value of the previously established MRLs. The analytical methods for monitoring tetracyclines in milk have a limit of quantification of 15μg/l, considerably lower than the present MRL for milk.

Accordingly, the Committee decided to double the recommended MRL values for edible tissues. The resulting MRLs are consistent with the available analytical methods and with good practice in the use of veterinary drugs. The Committee did not have information on the concentration of tetracyclines in milk that would interfere with the production of milk products such as yogurt and therefore made no change to the recommended MRL for milk.

The Committee recommended MRLs for chlortetracycline, oxytetracycline and tetracycline, expressed as parent drug, alone or in combination, as follows: 200μg/kg for muscle, 600μg/kg for liver and 1200μg/kg for kidney in cattle, pigs, sheep and poultry; 100μg/l for milk in cattle and sheep; and 400μg/kg for eggs (poultry).

At its thirty-sixth meeting, the Committee recommended an MRL for oxytetracycline of 100μg/kg for muscle of all species, including fish. At its present meeting, the Committee recommended increasing the MRL for fish to 200μg/kg. It further recommended that this MRL should remain temporary until data on the patterns of use of oxytetracycline in aquaculture can be evaluated.

At its forty-seventh meeting, the Committee recommended an MRL of 100μg/kg for oxytetracycline in muscle of giant tiger prawns (*Penaeus monodon*). In view of the available residue-depletion data and the ADI of 0–30μg/kg of body weight established at the present meeting,
the Committee recommended an MRL of 200μg/kg for oxytetracycline in muscle of giant tiger prawns, expressed as parent drug.

From the above recommended MRLs, the theoretical maximum daily intake of chlortetracycline, oxytetracycline and tetracycline, used alone or in combination, would be 370μg, based on a daily intake of 300g of muscle, 100g of liver, 50g each of kidney and fat, 100g of eggs and 1.5l of milk (Annex 1, reference 85).

3.3 Antiprotozoal agents

3.3.1 Diclazuril

Diclazuril, an anticoccidial drug, was previously evaluated at the forty-fifth meeting of the Committee (Annex 1, reference 119), when a temporary ADI of 0–20μg/kg of body weight was established. At that meeting, the Committee noted that, in a developmental study in rabbits, there was no evidence that exposure to diclazuril had been sufficient to enable the teratogenicity of the drug to be evaluated.

Toxicological data

The Committee considered additional information on the toxicity of diclazuril in non-pregnant female rabbits and on its toxicokinetics in pregnant and non-pregnant animals. The teratogenicity of the compound was also investigated. The studies were carried out according to appropriate standards for study protocol and conduct.

Two separate 2-week toxicity studies, including toxicokinetics, were performed in female rabbits as pilot studies to provide a dose-selection rationale for the definitive teratogenicity study. Diclazuril was administered by gavage to groups of rabbits (seven animals per group) at doses of 0, 80, 160 or 320mg/kg of body weight per day (first study) and 0, 320, 640 or 1280mg/kg of body weight per day (second study). Diclazuril was well tolerated at all dose levels.

In a study of the toxicokinetics of diclazuril, serial blood samples were collected over 24 hours from three rabbits from each dose group at day 10 (first study) or day 13 (second study). The plasma levels of diclazuril fluctuated only slightly throughout the sampling period after dosing. The plasma concentrations showed no clear dose-dependency and no linear increase with increasing dose. The results indicated a saturable mechanism for intestinal absorption of orally administered diclazuril, but demonstrated systemic exposure of the treated animals to the test compound.

In a developmental toxicity study, groups of 18 pregnant rabbits received diclazuril by gavage at doses of 0, 80, 320 or 1280mg/kg of
body weight per day from day 6 to day 18 of pregnancy. All animals were killed on day 28. Toxicokinetic studies performed on day 18 confirmed the previous findings of systemic exposure to diclazuril; plasma levels of diclazuril ranged from 4µg/ml at the lowest dose to 15µg/ml at the highest dose. Diclazuril was well tolerated by the dams at all doses, without any mortality. The only clinical signs observed were a slightly reduced food intake during the dosing period, reduced production of faeces, and a small number of abortions in animals dosed at 320 and 1280 mg/kg of body weight per day. The litter parameters revealed no drug-related adverse effects up to a dose of 320 mg/kg of body weight per day, whereas at the highest dose, resorptions and post-implantation losses were significantly increased. Fetal examination revealed a low incidence of drug-related minor abnormalities at 320 mg/kg of body weight per day. At 1280 mg/kg of body weight per day, diclazuril significantly increased the number of abnormalities and also induced malformations in approximately half of the fetuses. The Committee concluded that diclazuril is teratogenic in rabbits at high doses, whereas doses of up to 320 mg/kg of body weight per day are slightly fetotoxic but not teratogenic. The NOEL for developmental toxicity was 80 mg/kg of body weight per day. The Committee concluded that the additional studies complied with its previous request by showing enteric absorption and exposure of pregnant rabbits to doses of diclazuril which were sufficiently high to enable the teratogenicity of the drug to be assessed.

On the basis of these findings, the Committee considered the NOEL of 3 mg/kg of body weight per day in the 2-year toxicity/carcinogenicity study in mice reviewed at the forty-fifth meeting, based on histopathological changes in the liver, as the most appropriate toxicological end-point for establishing an ADI. An ADI of 0–30 µg/kg of body weight was established by applying a safety factor of 100 to the NOEL. This provides a margin of safety of at least 10 000 for the teratogenic effect of the compound in rabbits.

Residue data
At its forty-fifth meeting, the Committee recommended temporary MRLs for residues of diclazuril in certain food animals expressed as the parent compound (Annex 1, reference 119). The MRLs were temporary because the ADI was temporary.

No additional residue data were provided to the Committee for consideration at its present meeting. However, the Committee noted that the ADI established at its present meeting was higher than the temporary ADI allocated at the forty-fifth meeting and that the temporary MRLs recommended at the forty-fifth meeting already reflected good
practice in the use of this veterinary drug. The Committee therefore decided to delete the temporary qualification and to recommend the same MRLs as at its forty-fifth meeting.

The recommended MRLs are: 500µg/kg for muscle, 3000µg/kg for liver, 2000µg/kg for kidney and 1000µg/kg for fat, expressed as parent drug, in sheep and rabbits; 500µg/kg for muscle, 3000µg/kg for liver, 2000µg/kg for kidney and 1000µg/kg for fat/skin, expressed as parent drug, in poultry.

From the above MRLs, the theoretical maximum daily intake of diclazuril residues is 600µg, based on a daily food intake of 300g of muscle, 100g of liver, and 50g each of kidney and fat (Annex 1, reference 85).

### 3.3.2 Imidocarb

Imidocarb is a carbanilide derivative that has been used for over 20 years in the treatment of certain protozoal diseases, including babesiosis and anaplasmosis, in cattle, horses, sheep and dogs. Imidocarb had not been previously reviewed by the Committee.

*Toxicological data*

The Committee considered data from a range of toxicological studies on imidocarb, including the results of studies on its pharmacokinetics, metabolism, acute, short-term and long-term toxicity, carcinogenicity, reproductive toxicity and genotoxicity, as well as some special studies. Most of the studies were carried out using the dipropionate salt and the doses used were expressed as imidocarb base. Except for some of the genotoxicity assays, the studies carried out did not conform to contemporary standards for study protocol and conduct, but were reported in sufficient detail and were considered adequate for assessment.

The pharmacokinetics of imidocarb were investigated in mice, rats, dogs, monkeys and cattle. The dipropionate and dihydrochloride salts of imidocarb appeared to be poorly absorbed after oral administration to rats, but it was not possible to make any estimation of the extent of the bioavailability of the drug following oral administration from the data available. In cattle dosed subcutaneously, imidocarb was bound to the plasma proteins. The drug was excreted in both the urine and the faeces and excretion continued for several days in rodents and for at least 28 days in cattle.

In mice dosed intravenously with radiolabelled imidocarb and killed 3.5 hours later, over 90% of the residues in liver and kidney were unmetabolized imidocarb. Unmetabolized imidocarb also accounted
for 95% of the total radioactivity in urine. In cattle dosed subcutaneously with radiolabelled imidocarb, the parent compound accounted for most of the residues in urine and faeces and for 70–90% of the residues in edible tissues and milk. No evidence of metabolism was found in an in vitro study using sections of bovine liver, isolated hepatocytes and microsomal fractions.

Imidocarb dipropionate is moderately hazardous, with oral LD₅₀ values in the range of 650–720 mg/kg of body weight in mice and 450–1200 mg/kg of body weight in rats. The diacetate and dihydrochloride salts were of similar acute toxicity. Signs of acute toxicity were generally consistent with anticholinesterase activity.

Serum cholinesterase activity was depressed in a dose-related manner in goats which received single intramuscular doses in the range of 12–24 mg/kg of body weight. Cholinesterase activity in whole blood was decreased in calves given single intramuscular injections of 3.3 mg/kg of body weight.

Rats were given daily oral doses of imidocarb dihydrochloride at 0, 125, 250, 500, 750 or 1500 mg/kg of body weight per day for 3 months. All rats given 1500 mg/kg of body weight per day died. A cloudy swelling in the liver was observed on pathological examination of rats given 125 and 250 mg/kg of body weight per day. Histopathological examinations were not performed in animals given higher doses. A NOEL was not identified.

In a study conducted to identify appropriate dose levels of imidocarb dipropionate for a long-term toxicity study, rats were fed diets calculated to provide doses equal to 0, 26, 75 or 420 mg/kg of body weight per day for 90 days. Body-weight gain was reduced in both sexes given 420 mg/kg of body weight per day. Acetylcholinesterase activity measured in one-half of the brains from groups of rats given 420 mg/kg of body weight per day and killed after 7 and 13 weeks was not significantly different from the controls. Pathological changes attributable to treatment were limited to the liver in rats given 420 mg/kg of body weight per day; the changes consisted of mild stasis of the bile in the canaliculi of the liver in one of 15 males and four of 15 females. The NOEL in this study was 75 mg/kg of body weight per day, on the basis of reduced body-weight gain and liver toxicity at higher doses.

Groups of dogs (four male and four female) were given oral doses of 0, 5, 20 or 80 mg/kg of body weight per day of imidocarb dipropionate in gelatine capsules for 90 days. Signs of toxicity in the highest-dose group included recumbency, salivation, muscle fasciculation, ataxia
and splayed legs. All males and two out of four females given this dose died or were killed. Blood eosinophilia was also observed in animals dosed at 80mg/kg of body weight per day, together with increased serum alanine aminotransferase, aspartate aminotransferase and bilirubin. Similar but less severe changes were observed in the animals treated with 20mg/kg of body weight per day. Kidney, thyroid and adrenal weights were increased at a dose of 80mg/kg of body weight per day and histopathological changes were found in a range of tissues. In the kidney, these included fatty changes in the thick section of the loop of Henle and the distal convoluted tubules. The liver showed haemorrhagic necrosis, fatty changes and microgranularity or vacuolation of the hepatocytes. Similar but less severe hepatocellular changes were found in the livers of dogs given 20mg/kg of body weight per day. The NOEL was 5mg/kg of body weight per day, based on minor changes in haematology and clinical chemistry values and hepatocellular changes at higher dose levels.

In a combined long-term toxicity/carcinogenicity study, rats were fed diets calculated to provide 0, 15, 60 or 240mg of imidocarb dipropionate/kg of body weight per day for 2 years. Survival was adversely affected at the highest dose. Body-weight gain was significantly reduced in both sexes given 240mg/kg of body weight per day and was slightly reduced in females given 60mg/kg of body weight per day. Males given the highest dose showed evidence of anaemia. Changes in clinical chemistry values were transient and occurred mainly in the highest-dose group. Rats dosed at 60 and 240mg/kg of body weight per day consumed considerably more water than the controls and polyuria was observed in males given 240mg/kg of body weight per day. At the end of the study, the weights of kidneys from males given the highest dose were significantly increased. Dose-related histopathological changes were observed at doses of 60 and 240mg/kg of body weight per day; these included cystic distension of the renal tubules and glomeruli and dystrophic mineralization of the renal medulla. The NOEL for long-term toxicity was 15mg/kg of body weight per day, based on reduced body-weight gain, changes in some clinical chemistry values, and histopathological changes in the kidney. A gross pathological examination was carried out on all animals and all lesions were examined microscopically. However, a comprehensive pathological examination was made on only 10 rats of each sex in the control group and on 19 females and four males in the highest-dose group at the end of the study. A limited range of tissues was examined from the other animals and mammary glands were examined only when a gross lesion was found. At the highest dose significant increases in the incidence of multiple fibroadenomas of the
mammary gland in females and of multiple subcutaneous fibromas in males were observed. However, the Committee was unable to evaluate the significance of these findings, because of the high toxicity at this dose level, the poor survival rates, and the limited data on histopathology. No increases in the incidences of any other tumour types were reported.

The genotoxic properties of imidocarb dipropionate were investigated in a range of in vitro and in vivo assays. Negative results were obtained in in vitro assays for gene mutation in Salmonella typhimurium and in mammalian cells. Negative results were also obtained in a host-mediated assay in male mice. In two separate experiments, in the presence of metabolic activation, imidocarb induced polyploidy in human lymphocytes from peripheral blood in vitro. However, there was no evidence of induction of aneuploidy. Negative results were obtained in a micronucleus test in mouse bone marrow, and an in vivo chromosomal aberration assay in rat bone marrow. A dominant lethal assay in mice also gave negative results, although the dosing and mating schedules employed in this study were not in accordance with contemporary guidelines. The Committee concluded that imidocarb was unlikely to be genotoxic.

In a three-generation study of reproductive toxicity, rats were fed diets calculated to provide 0, 15, 45 or 135 mg of imidocarb dipropionate/kg of body weight per day. In the highest-dose group, maternal body-weight gain was decreased and the number of live births was reduced. The NOEL was 45 mg/kg of body weight per day for both maternal toxicity and fetotoxicity.

In a study of developmental toxicity in rats, there was no evidence of teratogenicity following administration of imidocarb dipropionate by gavage at 0, 19, 76 or 300 mg/kg of body weight per day from day 6 to day 18 of gestation. Between 20 and 25 dams from each dose group were killed on day 20 of gestation and their uterine contents examined. The remaining six dams in each dose group were allowed to deliver naturally and rear their offspring. Maternal body-weight gain was reduced at 300 mg/kg of body weight per day. The number of fetuses with bifid or H-shaped sternebrae was increased in dams receiving 76 and 300 mg/kg of body weight per day. There were no treatment-related effects on the growth or survival of the offspring postpartum. The NOEL for fetotoxicity was 19 mg/kg of body weight per day.

In a study of developmental toxicity in rabbits, there was no evidence of teratogenicity following administration of imidocarb dipropionate by gavage at 0, 5, 10, 20, 60 or 180 mg/kg of body weight per day from day 6 to day 16 of gestation. Severe maternal toxicity was observed at
60 and 180 mg/kg of body weight per day. All dams given the highest dose, and 12 out of 15 dams given 60 mg/kg of body weight per day, died. Post-implantation losses were increased and fetal weights were reduced in the group dosed at 60 mg/kg of body weight per day. The NOEL for maternal toxicity and fetotoxicity was 20 mg/kg of body weight per day.

An ADI of 0–10 μg/kg of body weight per day was established for imidocarb, based on the NOEL of 5 mg/kg of body weight per day in the 90-day study in dogs. A safety factor of 500 was used to compensate for the limited extent of the pathological and clinical chemistry investigations in this study, the absence of information concerning the potential for inhibition of erythrocyte and brain cholinesterase activity in this species, the lack of data concerning potential neurotoxicity, and the limited data on its carcinogenic potential.

Pharmacokinetic data

Rats. Several pharmacokinetic studies that did not comply with the requirements for good laboratory practice were conducted in rats, which received either [14C]imidocarb dipropionate or [14C]imidocarb hydrochloride. Absorption was poor in rats that received either of the imidocarb salts orally, while subcutaneous injection with the dihydrochloride resulted in a high level of residues at the injection site 7 days after treatment, with trace residues detectable in liver, kidney and muscle. In another study that failed to comply with the requirements for good laboratory practice, rats were dosed with unlabelled imidocarb at 10 mg/kg of body weight. Only about 19% of the dose was excreted as the parent compound within 78 hours, mainly in the urine. In rats given multiple doses by the oral or intraperitoneal route, residue levels were highest in kidney, followed by liver. In rats administered a single subcutaneous injection of 5 mg/kg of body weight, the residues in kidney and liver initially declined with a half-life of about 3 days, which increased subsequently to 7–8 days. There was evidence of significant binding of residues in liver. Following administration of imidocarb dipropionate at a dose of 5 mg/kg of body weight by stomach tube, either as a single treatment or once daily for 30 days, the residue levels were about 10 mg/kg in kidney, 1–2 mg/kg in liver and 0.5 mg/kg in muscle.

Mice. Several studies that did not comply with the requirements for good laboratory practice were also reported in mice. In mice administered [14C]imidocarb dihydrochloride intravenously, excretion of the drug was rapid, with residues appearing in the urine within 5 minutes of dosing; 90% of the drug was eliminated within 96 hours (55–65% in urine and 23–25% in faeces). In mice killed 3.5 hours after
treatment, 29% of the dose was found in liver and 6.8% in kidney, with over 90% of the extractable residues found in each tissue being the parent drug. The parent drug also accounted for 95% of the residues found in urine.

**Dogs and monkeys.** Several studies that did not comply with the requirements for good laboratory practice were conducted in dogs and monkeys. In one study, dogs received imidocarb dipropionate at a dose of 5 mg/kg of body weight as free base once daily by gavage for 30 days and were killed at 24 hours after the final dose. Imidocarb residues were distributed as follows: 98 mg/kg in liver, 7.3 mg/kg in kidney and <0.5 mg/kg in muscle. In another study in dogs given imidocarb dipropionate as an intravenous bolus dose of 4 mg/kg of body weight, the plasma half-life of imidocarb was 207 minutes, with approximately 80% of the dose being eliminated within 8 hours of treatment. In a study in monkeys which received imidocarb dipropionate orally at a dose of 5 mg/kg of body weight once daily for 30 days, imidocarb was distributed in the tissues as follows: 1.1 mg/kg in kidney, 1.0 mg/kg in liver and <0.5 mg/kg in muscle.

**Cattle.** Pharmacokinetic studies were conducted in cattle using [14C]imidocarb dipropionate and unlabelled drug. In a recent study that complied with the requirements for good laboratory practice, [14C]imidocarb dipropionate was administered to cattle as a single subcutaneous dose of 3 mg/kg of body weight. The concentration of the drug in blood reached a peak (1.3 mg/kg) within 1 hour of treatment and remained constant for 4 hours, then declined to <0.05 mg/kg over the following 3 days. Between 72% and 91% of the dose was bound to plasma proteins. Only 58% of the dose was eliminated up to 28 days after treatment; the residues were distributed between faeces and urine in a ratio of approximately 3:1. The parent compound accounted for most of the residues in urine, but up to 28% of the residues found in faecal samples at 4 days after dosing were accounted for by an unidentified metabolite. The same metabolite also accounted for 13% of the total residues in faecal samples collected 10 days after treatment, but was not detected in samples tested at days 2 and 6. In muscle, 89% of the total radioactive residues were readily extractable (without using enzyme digestion); in liver, kidney and milk, the values were 81%, 94% and 81%, respectively. The parent drug accounted for 88% of the total residues in muscle samples collected 28, 56 and 90 days after treatment and 68%, 88% and 77% of the total residues in liver, kidney and milk samples, respectively. There was no apparent reduction in the proportion of the total residues accounted for by the parent drug at the later sampling times. Other components present in extracts accounted for <10% of the total
radioactive residues, indicating that metabolism is not significant. Tissue binding was most significant in liver, which also had the lowest proportion of the total residues accounted for by the parent drug. This study confirmed the findings of earlier investigations (that did not comply with the requirements for good laboratory practice) in which [$^{14}$C]imidocarb dipropionate was administered intramuscularly to cattle. A recent *in vitro* study using bovine liver gave no indication of any metabolism of imidocarb in this tissue.

In studies that did not comply with the requirements for good laboratory practice, calves received unlabelled imidocarb dipropionate intravenously or as a pour-on formulation. The patterns of distribution and elimination of the drug were similar to those found in the recent study in cattle described above.

*Sheep.* Several studies were conducted in sheep, using methods that did not comply with the requirements for good laboratory practice. Intramuscular administration of [$^{14}$C]imidocarb dipropionate resulted in the distribution of residues throughout the central nervous system. Following intramuscular injection at a dose of 4.5mg/kg of body weight, peak plasma concentrations (7.9mg/l) were reached within about 4 hours, after which the concentrations declined slowly over 4 weeks to <0.1mg/l, following first-order kinetics. Significant binding to the plasma proteins was observed. There was no evidence of formation of metabolites in urine, bile, liver or kidney samples. Excretion of the drug in urine was high during the first 24 hours after treatment, declining rapidly afterwards.

*Residue data*

*Cattle.* A study was conducted in six dairy cattle and eight 9-month-old calves, which were treated with a single subcutaneous injection of a formulation containing [$^{14}$C]imidocarb dipropionate at a dose of 3mg/kg of body weight. The animals were killed at between 28 and 90 days after treatment. Table 3 shows the distribution of the total residues (determined by combustion) in the edible tissues. The results demonstrate that the elimination of residues is slow in all edible tissues. In milk, the concentration of total residues reached a maximum of 0.37mg/l at 24 hours after treatment and then declined to 0.10mg/l at day 3 and to 0.02mg/l at days 8–14.

In another study in cattle, which were treated with a single subcutaneous injection of unlabelled imidocarb dipropionate at a dose of 3mg/kg of body weight, the concentration of residues of the parent compound in muscle declined from 0.38mg/kg at day 28 to 0.37mg/kg at day 56 and to 0.21mg/kg at day 98. Residues were still detectable in
muscle (0.06 mg/kg) in animals slaughtered 224 days following treatment. The concentration of residues of the parent compound in liver declined from 5.4 mg/kg at day 14 to 0.12 mg/kg at day 224. These findings are consistent with those obtained in earlier studies in which various doses of unlabelled imidocarb dipropionate were administered by intramuscular or subcutaneous injection in single or multiple doses.

In two early studies that did not comply with the requirements for good laboratory practice, dairy cattle received a single intramuscular injection of imidocarb dipropionate at a dose of 3 mg/kg of body weight. The levels of residues of the parent compound in milk peaked at day 1 following treatment (0.60–0.79 mg/l) and then declined to 0.07–0.23 mg/l at day 3 and to <0.01 mg/l at days 7–28. Milk samples collected following administration of a second dose of 3 mg/kg of body weight after the day 28 sample was collected gave similar results.

**Sheep.** Several studies that did not comply with the requirements for good laboratory practice were reviewed. In one study, 12 sheep received a single intramuscular injection of imidocarb dipropionate at a dose of 1.2 mg/kg of body weight, which was repeated after 7 days. At 7 days following the second injection, the levels of residues in kidney ranged from 23 to 120 mg/kg, declining to 5.6–9.6 mg/kg at day 28. The levels of residues in liver ranged from 5.7 to 14 mg/kg at day 7 and from 0.9 to 3.1 mg/kg at day 28, while in muscle the levels were 1.1–1.2 mg/kg at day 7 and 0.1–0.4 mg/kg at day 28. The levels of residues in injection-site muscle were higher than in muscle from elsewhere on the animal, but lower than in kidney and liver at all sampling times.

In three lactating sheep that received imidocarb dipropionate intramuscularly at a dose of 4.5 mg/kg of body weight, the levels of residues in milk ranged from 4.5 to 5.6 mg/l in samples collected between 4 and

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<th>Withdrawal time (days)</th>
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* Injection site.
24 hours following treatment. The small number of animals involved and the methods and rates of dosing used limited the value of these studies in assessing the levels of residues.

**Analytical methods**

Recent studies that comply with the requirements for good laboratory practice have used an analytical method based on HPLC with ultraviolet detection at 245 nm following treatment of tissues with enzymic digestion to release residues, extraction and partitioning with solvents. The reported limits of detection of the method were 0.02 mg/kg for muscle, liver, kidney and fat and 0.01 mg/l for milk. The limits of quantification were 0.05 mg/kg for muscle and fat and 0.10 mg/kg for liver and kidney. The recoveries were 84.1% for muscle, 83.5% for liver, 92.6% for kidney, 95.9% for fat and 87.2% for milk.

An alternative method for the analysis of residues in bovine kidney has been reported using a weak cation-exchange solid-phase extraction cartridge, followed by HPLC with ultraviolet detection at 260 nm. This method has the disadvantage that it includes a partitioning step with chloroform, which has been categorized as an ozone-depleting solvent. The recovery was approximately 75% at 0.05–0.10 mg/kg and the limit of detection was 0.001 mg/kg.

The HPLC methods described above appear to be suitable for regulatory use, although additional validation for appropriate combinations of species and tissue matrices is required. In addition, alternatives to highly chlorinated solvents, such as chloroform, may be required.

**Choice of marker residue.** Imidocarb is the marker residue of choice in all tissues, although the study in cattle treated with radiolabelled imidocarb did reveal the presence of small amounts of metabolites that constituted <10% of the total radioactivity in some samples. This study was used to establish the proportion of the total residues accounted for by the parent drug. The proposed regulatory method includes an enzyme-digestion step, which was used in the analysis of tissue and milk samples from the study using the radiolabelled drug. The results were similar to those obtained when the same samples were analysed using the non-enzymic radiometric method.

**Maximum Residue Limits**

In recommending MRLs for imidocarb, the Committee took into account the following factors:

- An ADI of 0–10 µg/kg of body weight was established. This would result in a maximum ADI of 600 µg for a 60-kg person.
• The parent drug is the marker residue.
• Liver and muscle were considered to be the appropriate target tissues.
• On the basis of the residue-depletion study in cattle treated with radiolabelled imidocarb, the parent drug accounted for 88% of the total residues in muscle and kidney, 68% of the total residues in liver and 77% of the total residues in milk. Data were not available for fat, so a factor based on the lowest proportion in liver was applied.
• A suitable analytical method was available for analysis of imidocarb residues in edible tissues and milk.

The Committee recommended temporary MRLs for imidocarb of 300µg/kg for muscle, 2000µg/kg for liver, 1500µg/kg for kidney, 50µg/kg for fat and 50µg/l for milk in cattle, expressed as parent drug.

From these values for the MRLs, the theoretical maximum daily intake would be 582µg (Table 4).

The results of residue-depletion studies in which lactating and non-lactating cattle are given the recommended dose of unlabelled imidocarb by subcutaneous injection and the samples are analysed using the proposed regulatory method with enzymic digestion, are required for evaluation in 2001. The results of a residue-depletion study in sheep, in which the recommended dose and route of administration are used, would be required before MRLs could be considered for the edible tissues of this species.

Table 4
Theoretical maximum daily intake of imidocarb residues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Recommended MRL (µg/kg)</th>
<th>Estimate of total residues (µg/kg)</th>
<th>Theoretical maximum daily intakea (µg imidocarb equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>300</td>
<td>341c</td>
<td>102</td>
</tr>
<tr>
<td>Liver</td>
<td>2000</td>
<td>2941g</td>
<td>294</td>
</tr>
<tr>
<td>Kidney</td>
<td>1500</td>
<td>1705d</td>
<td>85</td>
</tr>
<tr>
<td>Fat</td>
<td>50</td>
<td>74e</td>
<td>4</td>
</tr>
<tr>
<td>Milk</td>
<td>50a</td>
<td>65f</td>
<td>97</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>582</td>
</tr>
</tbody>
</table>

a Expressed as parent drug.
b Based on a daily intake of 0.5 kg of meat made up of 300 g of muscle, 100 g of liver, and 50 g each of kidney and fat and 1.5 litres of milk.
c The marker residue accounted for 88% of the total residues in muscle and kidney.
d The marker residue accounted for 68% of the total residues in liver. Data were not available for fat, so a factor based on the lowest proportion of the total residues accounted for by the parent drug in liver was applied.
a Expressed in µg/l.
f The marker residue accounted for 77% of the total residues in milk.
3.3.3 *Nicarbazin*

Nicarbazin has been used in starter rations for broiler chickens for several decades as an aid in the prevention of faecal and intestinal coccidiosis. It may be used in combination with ionophore coccidiostats. The commercial product is an equimolar complex of N,N'-bis(4-nitrophenyl)urea and 4,6-dimethyl-2(1H)-pyrimidinone (also known as 4,4'-dinitrocarbanilide and 2-hydroxy-4,6-dimethylpyrimidine, respectively). The complex appears to be essential for the observed coccidiostatic properties of the drug. Nicarbazin has not been previously reviewed by the Committee.

*Toxicological data*

The Committee considered data from studies on toxicokinetics, acute, short-term and long-term toxicity, genotoxicity, reproductive toxicity and developmental toxicity. The studies were performed prior to the establishment of guidelines for the conduct of toxicological studies. Some of the reports were presented in sufficient detail for independent assessment and were considered to be of acceptable quality. An expert report was also available for consideration.

Following oral administration of nicarbazin in rats, measurement of drug levels in blood revealed the presence of low concentrations of the phenylurea component and high concentrations of the pyrimidinone. Excretion of the pyrimidinone component in the urine was considerably higher than of the phenylurea. On the basis of these results, it is likely that the pyrimidinone portion was absorbed to a greater extent and that most of the phenylurea was excreted in the faeces without absorption. No data were available on the metabolism of nicarbazin.

The acute oral toxicity of nicarbazin was low in rodents, the LD$_{50}$ values being >25000 mg/kg of body weight in mice and >10000 mg/kg of body weight in rats. The individual components also showed low acute oral toxicity, the LD$_{50}$ values in mice being 4000 and >18000 mg/kg of body weight for the pyrimidinone and phenylurea components, respectively.

A number of short-term studies with nicarbazin were available, but the reports were inadequate for detailed evaluation. They contained minimal details of the protocols used and limited data on toxicological findings and were often in the form of progress reports. These summaries reported that in rats, kidney damage in the form of crystalline deposits in the collecting tubules was seen at oral doses of 500 mg/kg of body weight per day and above. In dogs, bile duct proliferation was
the principal finding at an oral dose of 1600 mg/kg of body weight per day.

In both the 2-year study and the reproductive toxicity study in rats the highest dose chosen was 400 mg/kg of body weight per day. This dose was selected because of the formation of acetylated phenylurea with the resulting precipitation of crystals in the kidneys of rats administered oral doses of 500 mg/kg of body weight per day. In some toxicological studies, the test species were given the phenylurea and pyrimidinone components in a ratio of 3:1. This ratio was chosen since it was claimed that phenylurea and pyrimidinone are present in that ratio in the muscle of treated chickens. More recent data suggest that the ratio may be as high as 8:1.

A 2-year study was conducted in dogs fed diets containing the phenylurea and pyrimidinone components at 0 mg + 0 mg/kg of body weight, 60 mg + 20 mg/kg of body weight, 180 mg + 60 mg/kg of body weight or 600 mg + 200 mg/kg of body weight per day, respectively. Serum alanine aminotransferase was increased in several dogs and slight bile duct proliferation was observed in one dog at a dose of 600 mg + 200 mg/kg of body weight per day of the mixture of phenylurea and pyrimidinone. No other treatment-related effects were observed. The NOEL was 180 mg + 60 mg/kg of body weight per day of the mixture of phenylurea and pyrimidinone.

A 2-year study was carried out in rats fed diets containing the phenylurea and pyrimidinone components at 0 mg + 0 mg/kg of body weight, 50 mg + 17 mg/kg of body weight, 150 mg + 50 mg/kg of body weight or 300 mg + 100 mg/kg of body weight per day, respectively. There was no treatment-related toxicity and the incidence of tumours was unaffected. The NOEL was the highest dose, 300 mg + 100 mg/kg of body weight per day of the mixture of phenylurea and pyrimidinone.

Nicarbazin induced slight increases in the frequency of mutations in Salmonella typhimurium strains TA98 and TA1538 in one study but not in a second study. Mutations were not detected in S. typhimurium strains TA100, TA1535 or TA1537 or in E. coli WP2 and DNA damage was not induced in the rec assay. No other end-points were investigated. The examination of genotoxic potential was considered to be inadequate since studies were carried out only in bacteria.

A three-generation reproductive toxicity study was conducted in rats fed diets containing the phenylurea and pyrimidinone components at 0 mg + 0 mg/kg of body weight, 50 mg + 17 mg/kg of body weight,
150 mg + 50 mg/kg of body weight or 300 mg + 100 mg/kg of body weight per day. There were isolated occurrences of slightly reduced litter size at birth or depressed body-weight gain during lactation at the highest dose level. These effects were not observed in the majority of litters and showed no progression with the duration of the study. Therefore, the Committee concluded that nicarbazin had no significant effects on reproduction. The NOEL was the highest dose tested, 400 mg/kg of body weight per day of the 3:1 mixture of phenylurea and pyrimidinone.

Developmental toxicity was studied in rats given nicarbazin at 0, 70, 200 or 600 mg/kg of body weight per day (equimolar ratio of phenylurea:pyrimidinone) by gavage on days 7–17 of gestation. At 600 mg/kg of body weight per day, maternal food intake and body weight were depressed and seven out of 25 animals died. At this dose, the occurrence of lowered fetal body weight and reduced ossification suggested retarded fetal development. A number of fetal abnormalities were observed, in particular hydronephrosis and hyperplastic and bent ribs. The NOEL was 200 mg/kg of body weight per day, based on maternal and fetal toxicity; teratogenic effects were not observed.

The Committee noted the absence of certain toxicological studies in support of an ADI for nicarbazin. However, the other data available provided sufficient information to overcome the majority of these deficiencies. It was noted that nicarbazin has been used in veterinary medicine in many countries for over 40 years. On the basis of this long history of use and because use is restricted to starter rations in broiler chickens, the Committee considered that an ADI could be established.

The Committee established an ADI of 0–400 μg/kg of body weight for nicarbazin, based on the NOEL of 200 mg/kg of body weight per day in the developmental toxicity study in rats and a safety factor of 500. This safety factor was chosen to account for limitations in the available data.

**Pharmacokinetic data**

An excretion study was performed in chickens using nicarbazin, which was [14C]-radiolabelled in both the phenylurea and pyrimidinone portions of the molecule. The main pathway for excretion of the pyrimidinone portion of the complex was in the urine (>90%). This demonstrated that this moiety was well absorbed. It was also rapidly eliminated and by the third day after the last dose, 83% of the pyrimidinone had been eliminated. By contrast, the phenylurea portion of the nicarbazin complex was excreted predominantly (90%) in the faeces at a slower rate than the pyrimidinone, but the majority of
the radioactivity was recovered in the first 3 days after withdrawal of medication. The observed concentrations of the phenylurea portion in the urine were only 5–10% of those of the pyrimidinone portion, indicating that the kidney was not the major pathway of elimination.

**Metabolism data**

Broiler chickens were given feed containing 125 mg/kg of nicarbazin, [\(^{14}\text{C}\)]-labelled in both the phenylurea and pyrimidinone portions, for 7 days and killed in groups between day 2 and day 7. The concentrations of the [\(^{14}\text{C}\)]-labelled phenylurea portion were much higher in liver and kidney than in plasma and muscle. The concentrations of [\(^{14}\text{C}\)]-labelled pyrimidinone were highest in kidney, but they were not significantly lower in muscle, plasma and liver. The concentrations of the pyrimidinone portion of the nicarbazin complex in liver and kidney were about one-tenth of those of the phenylurea portion. The rapid elimination of the pyrimidinone portion of nicarbazin and the absence of detectable metabolites has led to an almost exclusive focus on the phenylurea portion of the complex in subsequent studies on metabolism and residue depletion.

In another study, broiler chickens were fed [\(^{14}\text{C}\)]nicarbazin, alone or with an ionophore, at a dose of 50 mg/kg for 5 days and then killed immediately. The metabolic pattern observed was the same with or without an accompanying ionophore. The phenylurea portion of the parent nicarbazin accounted for about 79% of the total radioactivity in the liver with about 10% of metabolite M3 (N,N'-4-acetylamino-4'-nitrodiphenylurea) and 2% of metabolite M1 (N,N'-bis-(4-acetylamino)phenyl)urea) also identified. In kidney the parent drug and metabolite M1 accounted for 6% and 13%, respectively, of the radioactive residues, with the remainder being non-extractable residues. From these data, N,N'-bis(4-nitrophenyl)urea was selected as the marker residue in all residue-depletion studies.

**Residue data**

Residue-depletion studies in which chickens were fed nicarbazin containing a [\(^{14}\text{C}\)]-radiolabel in both moieties at a dose of 125 mg/kg of feed for 3 days showed that both the parent drug and its metabolites were eliminated rapidly. An assay with a sensitivity of 0.003–0.004 mg/kg showed that all tissues were essentially devoid of radiolabelled residues from the pyrimidinone portion of nicarbazin by day 5 after withdrawal. The [\(^{14}\text{C}\)]-labelled residues emanating from the phenylurea portion were detectable only in liver at 5 days after withdrawal.
In a second study, chickens were fed nicarbazin with a $[^{14}\text{C}]$-radiolabel on either the phenylurea or the pyrimidinone portion of the molecular complex, in combination with ionophores, at a dose of 50 or 60 mg/kg of feed for 5 days. The chickens were killed immediately after the final dose. The pyrimidinone portion of the complex accounted for a much lower proportion of the total radioactive residues than did the phenylurea portion. The ratios of phenylurea to pyrimidinone residues, at the time of slaughter, were 8:1 in muscle, 53:1 in liver, 34:1 in kidney and 14:1 in fat. As in all the other studies, the phenylurea residue levels were highest in liver and kidney.

Another residue-depletion study was conducted in which nicarbazin was fed to chickens at a dose of 50 mg/kg of feed for 6 days. The drug contained a $[^{14}\text{C}]$-label in the phenylurea portion of the molecule, in combination with an ionophore. Total radioactivity was determined and the concentration of the phenylurea portion was measured by HPLC. The marker residue accounted for 65% of the total residues in muscle and 45%, 15% and 90% of those in liver, kidney and skin with adhering fat, respectively.

In a further study, chickens were fed diets containing nicarbazin at a dose of 125 mg/kg of feed daily for 49 days. The chickens were killed 24, 36, 48, 60 and 72 hours after withdrawal of the drug. The concentrations of $N,N'$-bis-(4-nitrophenyl)urea residue in muscle, liver and skin with adhering fat were determined by HPLC. At 36 hours after withdrawal, the residue levels were highest in liver (2.79–7.09 mg/kg), followed by skin with adhering fat (0.68–1.06 mg/kg) and muscle (0.37–0.88 mg/kg). By 72 hours after withdrawal, the residue levels in liver had declined to 0.90–3.39 mg/kg, while those in skin with adhering fat and muscle were <0.1–0.28 mg/kg and <0.1–0.21 mg/kg, respectively.

In an earlier, long-term feeding study, young chicks were fed a diet containing nicarbazin at a dose of 125 mg/kg of feed daily from 3 days of age until 44 days of age and were killed in groups 1, 3, 5, 7 and 9 days after the final dose. The highest concentration of residues of $N,N'$-bis-(4-nitrophenyl)urea occurred in liver, followed by kidney, skin with adhering fat and muscle, at all sampling times. The residue levels in kidney, skin with adhering fat and muscle declined to <0.2 mg/kg at 5 days after withdrawal and were about one-tenth of those in liver at all withdrawal times after day 1. The concentrations of marker residue in liver were 14–21 mg/kg at day 1, 3.0–9.4 mg/kg at day 3, 0.4–2.7 mg/kg at day 5, 0.14–0.59 mg/kg at day 7 and <0.1–0.12 mg/kg at day 9.
Analytical methods
Several procedures that use HPLC with ultraviolet detection for the determination of residues of the phenylurea portion of nicarbazin in chicken tissue are available. These methods appear to be suitable for the routine monitoring of nicarbazin residues down to a limit of detection of 0.02 mg/kg. A recent method for the analysis of the phenylurea portion of nicarbazin employed HPLC with ultraviolet detection, followed by HPLC-thermospray MS for confirmation of nicarbazin in chicken tissues. The overall average recovery of the phenylurea from fortified tissues was 83%. An analytical method validated in six laboratories in a trial by the United States Food and Drug Administration has a limit of quantification of 0.1 mg/kg and is suitable for routine monitoring of an MRL of 0.2 mg/kg in all tissues. Although nicarbazin is not approved for use in laying birds, suitable methods are available which can monitor residues in eggs with a detection limit of 0.005 mg/kg.

Maximum Residue Limits
In reaching its decision on MRLs for nicarbazin in broiler chickens (see Table 5), the Committee took the following factors into consideration:

- An ADI of 0–400 μg/kg of body weight was established. This would result in a maximum ADI of the parent drug and/or its equivalents of 24,000 μg for a 60-kg person.
- The limit of quantification of the analytical method is 0.1 mg/kg for all tissues.
- The parent drug is for use in broiler chickens only during the first 28 days after hatching.

Table 5
Theoretical maximum daily intake of nicarbazin residues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Recommended MRL (μg/kg)</th>
<th>Estimate of total residues (μg/kg)</th>
<th>Theoretical maximum daily intake (μg marker residue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>200</td>
<td>308(^b)</td>
<td>92</td>
</tr>
<tr>
<td>Liver</td>
<td>200</td>
<td>444(^a)</td>
<td>44</td>
</tr>
<tr>
<td>Kidney</td>
<td>200</td>
<td>1333(^a)</td>
<td>67</td>
</tr>
<tr>
<td>Fat/skin</td>
<td>200</td>
<td>222(^a)</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>214</td>
</tr>
</tbody>
</table>

\(^a\) Expressed as marker residue (N,N'-bis-(4-nitrophényl)urea).
\(^b\) Based on a daily intake of 0.5 kg of meat made up of 300 g of muscle, 100 g of liver, and 50 g each of kidney and fat.
\(^c\) The marker residue accounted for 65% of the total residues in muscle.
\(^d\) The marker residue accounted for 45% of the total residues in liver.
\(^e\) The marker residue accounted for 15% of the total residues in kidney.
\(^f\) The marker residue accounted for 90% of the total residues in fat.
• The marker residue is N,N'-bis-(4-nitrophenyl)urca.
• The marker residue accounts for 65% of the total residues in muscle and 45%, 15% and 90% of the total residues in liver, kidney and skin with adhering fat, respectively.
• The recommended MRLs are consistent with good practice in the use of veterinary drugs.

The Committee recommended MRLs of 200µg/kg for muscle, liver, kidney and skin with adhering fat in broiler chickens. From these MRLs, the theoretical maximum daily intake of residues as nicarbazin equivalents is 214µg, based on a daily food intake of 300g of muscle, 100g of liver, and 50g each of kidney and fat (Table 5).

3.4 Glucocorticosteroid

3.4.1 Dexamethasone

Dexamethasone was evaluated at the forty-second and forty-third meetings of the Committee (Annex 1, references I10 and I13). At the forty-second meeting, the Committee established an ADI of 0–0.015µg/kg of body weight for dexamethasone and recommended the following temporary MRLs: 0.5µg/kg for muscle, 2.5µg/kg for liver and 0.5µg/kg for kidney, expressed as parent drug, in cattle and pigs; and 0.3µg/l for cows’ milk, expressed as parent drug. At its forty-third meeting, the Committee recommended temporary MRLs for horses of 0.5µg/kg for muscle, 2.5µg/kg for liver and 0.5µg/kg for kidney, expressed as parent drug. At its forty-second meeting, the Committee noted that dexamethasone undergoes extensive metabolism. However, it also noted that the metabolites did not exhibit any biological activity and consequently proposed dexamethasone as the marker residue. The MRLs were designated as temporary because an adequate method to determine compliance with the MRL was not available.

Performance data were requested on the analytical method for evaluation at the forty-eighth meeting of the Committee, but no data were provided. The temporary MRLs for dexamethasone were withdrawn at that meeting due to lack of an adequate analytical method allowing enforcement of the MRLs. At its present meeting, the Committee reviewed documentation on an HPLC–MS method for the control of dexamethasone residues in tissues and milk.

Analytical methods

HPLC methods based on ultraviolet detection were considered unsuitable for analysis of dexamethasone residues at concentrations of <1 µg/kg. Although a method has been described for analysis of dexamethasone residues in milk samples at concentrations of 0.1 µg/l by gas chromatography–MS using monitoring of negative chemical ions,
attempts to apply this method to other food commodities failed. Immunoassays were considered to meet the required limits of detection, but other technical problems were encountered. Recently, a liquid chromatography–thermospray MS (TS–LC/MS) method was developed for the analysis of dexamethasone residues. The studies were performed in accordance with the requirements of good laboratory practice.

The TS–LC/MS method requires high-quality laboratories to maintain the complex and expensive equipment and skilled operating personnel. Failure to maintain instrument performance may adversely affect the reproducibility of the method. The transferability of such a method is questionable and this limits its use as a regulatory method.

The preparation of samples of tissue, milk and serum was performed using liquid/liquid extraction. The sample was homogenized in buffer, extracted and purified and then transferred to the TS–LC/MS system.

The TS–LC/MS method involves gradient elution using a reversed-phase column. The analytes are identified by their retention times. The chromatograms provided with the information from the sponsor showed some apparent instabilities in retention time, but did not offer any explanation for this phenomenon.

A large variation in detector response was reported to occur during the analysis. Non-specific interferences were encountered occasionally, requiring adjustments to be made to the concentrations measured. Such adjustments are possible with fortified samples, but cannot be done accurately with samples containing incurred residues. Therefore, calculation of quantitative results with samples containing incurred residues may not be accurate.

Dexamethasone is identified on the basis of its retention time and the presence of a single 333 m/z ion. Methyl prednisolone (315 m/z) was chosen as the internal standard for quantification of dexamethasone residues. Prednisolone, cortisone, methylprednisolone, triamcinolone, flumethasone and isoflupredone did not interfere with the analysis of dexamethasone. However, betamethasone, an isomer of dexamethasone, was found to elute together with dexamethasone and to possess the same 333 m/z ion. Consequently, the method cannot be used to obtain an unequivocal identification of dexamethasone, if the 333 m/z ion is used.

*Validation of the method.* The linearity of the detector response was determined using standards with concentrations ranging from 0.25 to 10μg/l for milk and 0.5 to 20μg/kg for tissues and plasma. The linearity was considered acceptable when the coefficient of correlation exceeded 0.98. The recovery and accuracy of the method were
determined by analysing five replicate samples fortified with dexamethasone at the limit of quantification, twice the limit of quantification, and 10 times the limit of quantification. The method failed to report absolute recoveries. The criteria set by the Codex Committee on Residues of Veterinary Drugs in Foods (5) for accuracy and precision were used. The reported limits of quantification were 0.5 µg/kg for muscle, kidney and fat in cattle, pigs and horses. The reported limits of quantification for liver were 0.5, 1.0 and 1.0 µg/kg in cattle, pigs and horses, respectively. The limits of quantification for pig skin and cows' milk were 0.5 µg/kg and 0.25 µg/l, respectively.

The Committee concluded that the analytical method did not meet the required performance criteria for the identification and quantification of incurred residues of dexamethasone in tissues. Therefore, the method was not considered to be suitable for the analysis of dexamethasone residues for regulatory purposes. In the absence of an acceptable analytical method for monitoring purposes, the Committee was unable to recommend MRLs for dexamethasone.

3.5 Production aid

3.5.1 Recombinant bovine somatotropins (rbSTs)

Four analogues of bovine somatotropin (bST) that are produced by recombinant DNA techniques (rbSTs) (somagrobove, sometribove, somavubove and somidobove) were previously evaluated by the Committee at its fortieth meeting (Annex 1, reference 104). At that time, the Committee established an ADI and MRLs “not specified” ¹ for these four rbSTs. The term “not specified” was used because of the lack of oral activity of rbSTs and insulin-like growth factor I (IGF-I) and the low levels and non-toxic nature of the residues of these compounds, which result in an extremely large margin of safety for humans consuming meat and dairy products from rbST-treated cows. When considering the adoption of these recommended MRLs at its Twenty-second Session in 1997 (8), the Codex Alimentarius

¹ ADI "not specified" means that available data on the toxicity and intake of the veterinary drug indicate a large margin of safety for consumption of residues in food when the drug is used according to good practice in the use of veterinary drugs. For that reason, and for the reasons stated in the individual evaluation, the Committee concluded that use of the veterinary drug does not represent a hazard to human health and that there is no need to specify a numerical ADI.

² MRL "not specified" means that available data on the identity and concentration of residues of the veterinary drug in animal tissues indicate a wide margin of safety for consumption of residues in food when the drug is used according to good practice in the use of veterinary drugs. For that reason, and for the reasons stated in the individual evaluation, the Committee concluded that the presence of drug residues in the named animal product does not present a health concern and that there is no need to specify a numerical MRL.
Commission postponed its decision until a re-evaluation of the rbSTs had been made by the Expert Committee, based on scientific information that had become available since the previous evaluation.

At the present meeting, the Committee considered information submitted by organizations and individuals relating to the following concerns:

- The increased use of antibiotics to treat mastitis in cows, which leads to a higher rate of "violative" drug residues (i.e. residues exceeding regulatory limits) in milk, possibly because of an increase in the incidence of mastitis in cows treated with rbSTs.
- The possibility that increased levels of IGF-I in the milk of cows treated with rbSTs might lead to increased cell division and growth of tumours in humans.
- The potential effect of rbSTs on the expression of certain viruses in cattle, particularly retroviruses.
- The possibility that the incubation period of bovine spongiform encephalopathy (BSE) is shortened due to an increase in the production of pathogenic prion proteins induced by IGF-I.
- The possibility that early exposure of human neonates to milk from rbST-treated cows increases their risk of developing insulin-dependent diabetes mellitus.

**Use of antibiotics**

After reviewing the available information, the Committee decided that the risk of mastitis induced by rbSTs was an issue of animal health that is not within its terms of reference. However, the possible increased use of antibiotics in cows treated with rbSTs was considered.

A post-approval monitoring programme has been established in the United States to address the following areas:

- The incidence of mastitis and its effects on the health of dairy herds (not within the terms of reference of the Committee).
- Any treatment with medications of rbST-treated cows in a study of 28 herds (not within the terms of reference of the Committee).
- The percentage of milk discarded because of the levels of violative drug residues it contains in key dairy states that represent at least 50% of milk production in the USA.

In New York state, the percentage of milk samples discarded as a result of testing for residues of antibiotics did not change significantly after the introduction of rbSTs. In other states, a small but statistically significant increase was observed in 1995, which coincided with a change to a more sensitive testing method. The Committee concluded
that the use of rbSTs would not result in a higher risk to human health due to the use of antibiotics to treat mastitis and that the increased potential for the presence of drug residues in milk could be managed by practices currently in use by the dairy industry and by following the drug manufacturers’ directions for use.

*IGF-I levels in milk and tissues*

IGF-I is a normal component of milk and is found in abundance in a variety of body fluids (see Table 6).

The presence and concentrations of IGF-I in milk were at the centre of much of the scientific discussion in the original review of bovine somatotropins undertaken at the fortieth meeting of the Committee and in submissions to the present meeting. The information that was reviewed is summarized in FAO Food and Nutrition Paper No. 41/5 (Annex 1, reference 106). The concentrations of IGF-I in milk are variable and have been shown to depend on stage of lactation, nutritional state and age.

The methods that are used for assaying IGF-I were considered by the Committee. Although incomplete removal of IGF-binding proteins or variation in the reference standard and extraction methods might influence reported values, these factors were not perceived to materially alter any conclusions. The relatively high values previously reported in milk were considered to reflect inadequate extraction procedures.

<table>
<thead>
<tr>
<th>Fluid</th>
<th>IGF-I concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human milk</td>
<td></td>
</tr>
<tr>
<td>colostrum</td>
<td>8-24</td>
</tr>
<tr>
<td>Cows’ milk (bulk milk)</td>
<td></td>
</tr>
<tr>
<td>untreated</td>
<td>1-9</td>
</tr>
<tr>
<td>treated with rbSTs</td>
<td>1-13</td>
</tr>
<tr>
<td>Human plasma</td>
<td></td>
</tr>
<tr>
<td>children</td>
<td>17-250</td>
</tr>
<tr>
<td>adolescents</td>
<td>180-780</td>
</tr>
<tr>
<td>adults</td>
<td>120-460</td>
</tr>
<tr>
<td>Human gastrointestinal secretions</td>
<td></td>
</tr>
<tr>
<td>saliva</td>
<td>6.8</td>
</tr>
<tr>
<td>gastric juice</td>
<td>26</td>
</tr>
<tr>
<td>pancreatic juice</td>
<td>27</td>
</tr>
<tr>
<td>bile</td>
<td>6.8</td>
</tr>
<tr>
<td>jejunal chyme</td>
<td>160</td>
</tr>
</tbody>
</table>

* The estimated total daily production in adults is 107 ng.
EVALUATION OF CERTAIN VETERINARY
DRUG RESIDUES IN FOOD

Fiftieth report of the Joint FAO/WHO Expert
Committee on Food Additives

CORRIGENDUM

Page 76, Table 6, footnote a:

Delete The estimated total daily production in adults is 107 ng.

Insert The estimated total daily production in adults is $10^7$ ng.
Since the previous evaluation, very few additional data on residues have appeared in the literature or in reports provided by interested parties. However, the manufacturer of sometribove submitted additional information on levels of IGF-I in milk available from retailers after the approval of rbSTs in the United States. The results showed no difference in the IGF-I concentrations between labelled milk (certified to be derived from cows not treated with rbSTs) and unlabelled milk. However, the percentage of unlabelled milk derived from cows receiving rbSTs was not specified.

Concerns have been expressed that any increase of IGF-I in milk induced by rbSTs could contribute to the endogenous levels of IGF-I in the gastrointestinal tract (if it is not degraded) and in serum (if absorption occurs). A recent study in rats confirmed that orally administered IGF-I is rapidly degraded in the gastrointestinal tract. However, casein has been shown to reduce the rate of degradation of IGF-I in the gastrointestinal tract. It was postulated that reduced degradation of IGF-I in the gastrointestinal tract leads to increased serum levels (as shown in a study in rats) as well as to prolonged exposure to IGF-I in the gut. The Committee noted that oral administration of high doses of IGF-I in milk replacer to newborn calves and piglets for 7 days did not increase plasma concentrations of IGF-I, indicating that significant absorption is unlikely to occur under physiological conditions.

If the decreased rate of degradation observed in the small intestine of rats in the presence of casein is taken into account, the IGF-I levels would be likely to deplete to less than 5% of their initial values within 2 hours, indicating that IGF-I in milk would not be expected to contribute to the normal IGF-I levels in the large intestine.

If 1.5l of milk are ingested per day, the average amount of IGF-I ingested would be 6000 ng in milk from untreated cows, assuming an IGF-I concentration of 4 ng/ml, and 9000 ng in milk from rbST-treated cows, assuming an average concentration of 6 ng/ml. It has been calculated that IGF-I production in gastrointestinal secretions in humans amounts to about 380 000 ng/day. Therefore, the additional amount of IGF-I consumed in 1.5l of milk from rbST-treated cows as compared with milk from untreated cows is only about 0.8% of that produced in gastrointestinal secretions.

The total amount of IGF-I in serum has been calculated to range from approximately 50 000 to 1220 000 ng, depending on age. The total daily production of IGF-I in adult humans has been estimated to be $10^7$ ng. Therefore, the amount of IGF-I ingested in milk from rbST-treated cows will be less than 0.09% of the amount produced daily in
adults. Even if the total amount of IGF-I from milk were absorbed, the additional amount would be negligible.

In recent studies, sustained-release rbSTs were administered to cattle once every 2 weeks for a total of 20 weeks. The levels of rbSTs and IGF-I in tissues were measured 2 weeks after the final administration of rbSTs. No significant increases in levels of rbSTs or IGF-I were observed.

The Committee concluded that any increase in the amount of IGF-I in human tissues resulting from consumption of milk from cows treated with rbSTs would be several orders of magnitude lower than the amounts produced physiologically in the gastrointestinal tract as well as in other parts of the body. Thus, the Committee concluded that the levels of IGF-I would not increase either locally in the gut or systemically. Consequently, the potential for IGF-I to promote tumours would not increase when milk from cows treated with rbSTs is consumed, resulting in no appreciable risk for consumers.

**Expression of retroviruses**

Concerns that the treatment of cattle with rbSTs would increase the expression of retroviruses, including the lentivirus bovine leukaemia virus, were addressed by experiments in goats injected with monocytes infected *in vitro* with the lentivirus caprine arthritis encephalitis virus. The infectivity was not increased when measured by numbers of infected cells and there was no evidence of increased activity of reverse transcriptase. These studies provided no evidence that rbSTs affect the expression of lentiviruses such as bovine leukaemia virus. Furthermore, it has been shown that bovine leukaemia virus is destroyed in simulated pasteurization conditions by heating milk to 60°C for 30 seconds. In addition, there was no evidence of any susceptibility or responses in humans to ruminant retroviruses.

**Expression of prion proteins**

Concerns have been expressed that treatment of cows with rbSTs could shorten the incubation period for bovine spongiform encephalopathy (BSE). This hypothesis is based on results obtained *in vitro* in a neuronal cell line, which indicated an increased formation of mRNA for prion proteins in response to IGF-I. Furthermore, in transgenic mice harbouring multiple copies of the gene that codes for prion proteins, an increased formation of prion protein shortened the incubation period for scrapie. However, no data were available that directly address whether IGF-I or rbSTs increase the formation of normal prion protein or its pathogenic protease-resistant mutant in the brain of cattle. The Committee considered the possibility
of a link between treatment with rbSTs and BSE to be highly speculative.

Risk of insulin-dependent diabetes mellitus
It has been shown that exposure of neonates to cows' milk increases the risk of insulin-dependent diabetes mellitus approximately 1.5-fold. At its present meeting, the Committee considered whether exposure of human neonates to milk from cows treated with rbSTs further increases this risk. The Committee concluded that, because the composition of the milk of rbST-treated cows is unchanged, it is not expected to represent an additional risk to the development of insulin-dependent diabetes mellitus.

Conclusions
The Committee concluded that rbSTs can be used without any appreciable health risk to consumers. This conclusion was based on the following factors:

— the insignificant changes in the quantities of milk discarded due to the detection of antibiotic residues during testing after the introduction of rbSTs into commercial use;
— the low levels of residues of rbSTs and IGF-I in milk;
— the degradation of IGF-I in the gut and its naturally high levels in the gastrointestinal secretions;
— the extremely low levels of IGF-I ingested from milk when compared to endogenous production;
— the lack of evidence that rbSTs stimulate the expression of retroviruses;
— the lack of information directly linking treatment with rbSTs and BSE; and
— the absence of significant changes in the composition of milk from cows treated with rbSTs that may contribute to the additional risk of development of insulin-dependent diabetes mellitus.

The Committee reaffirmed its previous ADI\(^1\) and MRLs\(^2\) “not specified” for somagrebove, sometribove, somavubove and somidobove.

3.6 Tranquillizing agent
3.6.1 Azaperone
Azaperone is a butyrophenone neuroleptic tranquillizer which had previously been evaluated at the thirty-eighth and forty-third

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\(^1\) See footnote 1 on p. 74.
\(^2\) See footnote 2 on p. 74.

79
meetings of the Committee (Annex 1, references 97 and 113). At the forty-third meeting, a temporary ADI of 0–3µg/kg of body weight was established and the Committee requested the following information:

1. Results of studies to determine the genotoxic potential of the metabolites of azaperone, which have been reported to be mutagenic in *Salmonella* spp.
2. Evidence to support the claim that azaperone or its degradation products are not structurally similar to known carcinogens.
3. Results of a study to assess the effects of azaperone on reproduction and fertility in male laboratory animals.

*Toxicological data*

At its present meeting, the Committee considered new data to address the above-mentioned issues. The relevant studies were carried out according to appropriate standards for study conduct and protocol.

A study was performed to address the issue of the genotoxic potential of azaperone metabolites. The metabolism of azaperone was studied by incubating various concentrations of radiolabelled azaperone with liver S-9 fractions prepared from rats treated with Aroclor (a mixture of polychlorinated biphenyls) to determine what metabolites are formed under the conditions used in the Ames test. The metabolites were isolated and identified and shown to be similar to the spectrum of metabolites produced in the target species (pigs). The Committee therefore concluded that the results of the Ames tests using azaperone that were reviewed at the thirty-eighth and forty-third meetings were adequate to assess the genotoxic potential of azaperone and its metabolites in bacteria. Taking into account all of the genotoxicity studies performed, the Committee concluded that azaperone was unlikely to be genotoxic.

The Committee reviewed a brief report that discussed the lack of structural similarity of azaperone and its metabolites to known carcinogens. It was noted that azaperone, a neuroleptic of the butyrophenone class, and its metabolites do not contain any reactive subgroups. The argument was further reinforced by the lack of evidence of genotoxicity of azaperone in a battery of studies. The Committee concluded that azaperone was not likely to be carcinogenic.

Studies were conducted to evaluate the effects of azaperone on reproduction and fertility in male rats. Azaperone was administered to male rats daily by gavage for 74 days at dosages of 0, 5, 20 or 80mg/kg of body weight per day. Signs of toxicity including severe sedation, ptosis, and decreases in body weights and feed consumption were observed at the highest dose throughout the treatment period. Slight
to moderate sedation and ptosis were seen at the dose of 20mg/kg of body weight. Copulation and fertility rates and pre-coital intervals were comparable in all groups. There were no adverse effects on male fertility or on the development of embryos or fetuses in untreated females bred to treated males. The NOEL for paternal toxicity was 5mg/kg of body weight per day.

The Committee concluded that the pharmacological effects of azaperone were the most relevant for the determination of an ADI. An ADI of 0–6μg/kg of body weight was therefore established, based on the NOEL of 630μg/kg of body weight for pharmacological activity following oral administration in dogs in the study considered at the forty-third meeting of the Committee and a safety factor of 100.

*Residue data*

At its forty-third meeting, the Committee had recommended temporary MRLs for azaperone in pigs of 60μg/kg for muscle and fat, and 100μg/kg for liver and kidney, expressed as the sum of the concentrations of azaperone and azaperol (Annex 1, reference 113).

No new residue data were provided to the Committee for consideration at the present meeting. However, the Committee noted that the temporary MRLs established at the forty-third meeting were consistent with good practice in the use of this veterinary drug. It therefore decided to delete the temporary qualification and to recommend the same MRLs as at its forty-third meeting.

4. **Recommendations**

1. Recommendations relating to specific veterinary drugs, including ADIs and MRLs, are given in section 3 and Annex 2.

2. In view of the large number of veterinary drugs requiring evaluation, meetings of the Joint FAO/WHO Expert Committee on Food Additives should be held annually for this purpose.

3. The Committee recommended that FAO and WHO consider appropriate procedures for harmonizing the evaluation policies of the Joint FAO/WHO Expert Committee on Food Additives and the Joint FAO/WHO Meeting on Pesticide Residues.

**Acknowledgement**

The Expert Committee wishes to thank Mrs E. Heseltine, Saint-Léon-sur-Vézère, France, for her assistance in the preparation of this report.
References


Annex 1
Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives


113. Evaluation of certain veterinary drug residues in food (Forty-third report of the


Annex 2

Recommendations on compounds on the agenda and further toxicological studies and other information required

Anthelmintic agents

*Eprinomectin*

**ADI:** 0–10 µg/kg of body weight.

Residue definition: Eprinomectin B₁₄.

<table>
<thead>
<tr>
<th>Species</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
<th>Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>100</td>
<td>2000</td>
<td>300</td>
<td>250</td>
<td>20</td>
</tr>
</tbody>
</table>

*a* Expressed in µg/l.

*Febantel, fenbendazole and oxfendazole*

**ADI:** 0–7 µg/kg of body weight (group ADI for febantel, fenbendazole and oxfendazole).

Residue definition: Sum of fenbendazole, oxfendazole and oxfendazole sulfone, expressed as oxfendazole sulfone equivalents.

<table>
<thead>
<tr>
<th>Species</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
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</thead>
<tbody>
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<td>100</td>
<td>100</td>
<td>100</td>
</tr>
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<td>Horses</td>
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</tr>
<tr>
<td>Pigs</td>
<td>100</td>
<td>500</td>
<td>100</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>Sheep</td>
<td>100</td>
<td>500</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Goats</td>
<td>100</td>
<td>500</td>
<td>100</td>
<td>100</td>
<td>—</td>
</tr>
</tbody>
</table>

*a* Expressed in µg/l.

*Moxidectin*

**ADI:** 0–2 µg/kg of body weight (established at the forty-fifth meeting of the Committee (WHO Technical Report Series, No. 864, 1996)).

Residue definition: Moxidectin.
<table>
<thead>
<tr>
<th>Species</th>
<th>Recommended MRLs (µg/kg)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td>Cattle</td>
<td>20b</td>
</tr>
<tr>
<td>Sheep</td>
<td>50</td>
</tr>
<tr>
<td>Deer</td>
<td>20d</td>
</tr>
</tbody>
</table>

* These MRLs were recommended at the forty-fifth meeting of the Committee (WHO Technical Report Series, No. 884, 1996), except for the MRL for sheep muscle, which was recommended at the forty-seventh meeting (WHO Technical Report Series, No. 876, 1998).

At the forty-fifth meeting (WHO Technical Report Series, No. 884, 1996), the Committee noted the very high concentrations and great variation in the level of residues at the injection site in cattle over a 49-day period after dosing.

At the forty-fifth meeting (WHO Technical Report Series, No. 864, 1996), the Committee noted that the sponsors do not intend to make the drug available for use in lactating cows and cows in late pregnancy. Thus, residues in milk should not be taken into account.

Final MRLs were recommended at the present meeting of the Committee.

**Antimicrobial agents**

**Gentamicin**

ADI: 0–20 µg/kg of body weight.

Residue definition: Gentamicin.

<table>
<thead>
<tr>
<th>Species</th>
<th>Recommended MRLs (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td>Cattle</td>
<td>100</td>
</tr>
<tr>
<td>Pigs</td>
<td>100</td>
</tr>
</tbody>
</table>

* Expressed in µg/l.

**Procaine benzylpenicillin**

ADI: Daily intake of residues of benzylpenicillin and procaine benzylpenicillin should be kept below 30 µg of the penicillin moiety.

Residue definition: Benzylpenicillin.

<table>
<thead>
<tr>
<th>Species</th>
<th>Recommended MRLs (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td>All species</td>
<td>50</td>
</tr>
</tbody>
</table>

* Expressed in µg/l.

**Sarafloxacin**

ADI: 0–0.3 µg/kg of body weight.

Residue definition: Sarafloxacin.
Species | Recommended MRLs (µg/kg) | Muscle | Liver | Kidney | Fat |
---|---|---|---|---|---|
Turkeys | 10 | 80 | 80 | 20<sup>a</sup> |
Chickens | 10 | 80 | 80 | 20<sup>a</sup> |

<sup>a</sup> Fat/skin in normal proportions.

**Spectinomycin**

ADI: 0–40µg/kg of body weight (established at the forty-second meeting of the Committee (WHO Technical Report Series, No. 851, 1995)).

Residue definition: Spectinomycin.

Species | Recommended MRLs (µg/kg) | Muscle | Liver | Kidney | Fat | Milk<sup>a</sup> |
---|---|---|---|---|---|---|
Cattle | 500 | 2000 | 5000 | 2000 | 200 | — |
Pigs | 500 | 2000 | 5000 | 2000 | — | — |
Sheep | 500 | 2000 | 5000 | 2000 | — | 2000 |
Chickens | 500 | 2000 | 5000 | 2000 | — | — |

<sup>a</sup> Expressed in µg/l.

**Chlortetracycline, oxytetracycline and tetracycline**

ADI: 0–30µg/kg of body weight (group ADI for tetracycline, oxytetracycline and chlortetracycline).

Residue definition: Parent drug.

Species | Recommended MRLs<sup>ab</sup> (µg/kg) | Muscle | Liver | Kidney | Fat | Milk<sup>b</sup> |
---|---|---|---|---|---|---|
Cattle | 200 | 600 | 1200 | —<sup>c</sup> | 100 | — |
Pigs | 200 | 600 | 1200 | —<sup>c</sup> | — | — |
Sheep | 200 | 600 | 1200 | —<sup>c</sup> | 100 | — |
Poultry | 200 | 600 | 1200 | —<sup>c</sup> | — | 400 |
Fish<sup>d</sup> | 200 | — | — | — | — | — |
Giant tiger prawn<sup>ed</sup> | 200 | — | — | — | — | — |

<sup>a</sup> Singly or in combination.
<sup>b</sup> Expressed in µg/l.
<sup>c</sup> At its forty-seventh meeting (WHO Technical Report Series, No. 876, 1998), the Committee recommended that the MRL for oxytetracycline in fat be withdrawn, and decided that the MRLs for chlortetracycline and tetracycline in fat were not required.
<sup>d</sup> Applies only to oxytetracycline.
<sup>ed</sup> Temporary MRL, pending evaluation of the pattern of use of oxytetracycline in aquaculture.
Antiprotazoal agents

Diclazuril

ADI: 0–30μg/kg of body weight.

Residue definition: Diclazuril.

<table>
<thead>
<tr>
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<th>Recommended MRLs (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td>Sheep</td>
<td>500</td>
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<tr>
<td>Poultry</td>
<td>500</td>
</tr>
<tr>
<td>Rabbits</td>
<td>500</td>
</tr>
</tbody>
</table>

* Fat/skin in normal proportions.

Imidocarb

ADI: 0–10μg/kg of body weight.

Residue definition: Imidocarb.

<table>
<thead>
<tr>
<th>Species</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td>Cattle</td>
<td>300</td>
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</tbody>
</table>

* Temporary MRLs, pending the receipt of the results of residue-depletion studies in which lactating and non-lactating cattle are given the recommended dose of unlabelled imidocarb by subcutaneous injection and samples are analysed using the proposed regulatory method with enzymic digestion. These results are required for evaluation in 2001. The results of a residue-depletion study in sheep, in which the recommended dose and route of administration are used, would be required before MRLs could be considered for the edible tissues of this species.

Nicarbazin

ADI: 0–400μg/kg of body weight.

Residue definition: N,N'-bis-(4-nitrophenyl)urea.

<table>
<thead>
<tr>
<th>Species</th>
<th>Recommended MRLs (μg/kg)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td>Chickens (broilers)</td>
<td>200</td>
</tr>
</tbody>
</table>

* Fat/skin in normal proportions.

Glucocorticosteroid

Dexamethasone

ADI: 0–0.015μg/kg of body weight (established at the forty-second meeting of the Committee (WHO Technical Report Series, No. 851, 1995)).
Residue definition: Dexamethasone. At its forty-second and forty-third meetings (WHO Technical Report Series, No. 851, 1995; WHO Technical Report Series, No. 855, 1995), the Committee recommended temporary MRLs of 0.5μg/kg for muscle, 2.5μg/kg for liver and 0.5μg/kg for kidney in cattle, horses and pigs and 0.3μg/l for cows' milk, based on an ADI of 0–0.015μg/kg of body weight. The MRLs were temporary because there was no adequate method to determine compliance with the MRLs. The Committee requested performance data on the analytical method for evaluation at its forty-eighth meeting (WHO Technical Report Series, No. 879, 1998), but no data were received. The temporary MRLs were not extended due to lack of an adequate analytical method for regulatory monitoring. At its present meeting, the Committee reviewed the documentation on an HPLC–MS method for measuring dexamethasone residues in tissues and milk. The Committee concluded that the proposed method does not meet the required performance characteristics for identification and quantification of incurred residues of dexamethasone in tissues. In the absence of an acceptable analytical method for monitoring purposes, the Committee was unable to recommend MRLs for dexamethasone.

Production aid

Recombinant bovine somatotropins (rbSTs)

ADI: “Not specified”¹ (applies to somagrebove, sometribove, somavubove and somidobove).

¹ ADI “not specified” means that available data on the toxicity and intake of the veterinary drug indicate a large margin of safety for consumption of residues in food when the drug is used according to good practice in the use of veterinary drugs. For that reason, and for the reasons stated in the individual evaluation, the Committee concluded that use of the veterinary drug does not represent a hazard to human health and that there is no need to specify a numerical ADI.
MRLs: “Not specified”¹ for edible tissues and milk in cattle (applies to somagreve, sometribove, somavubove and somidobove).

**Tranquilizing agent**

**Azaperone**

**ADI:** 0–6 µg/kg of body weight.

**Residue definition:** Sum of concentrations of azaperone and azaperol.

<table>
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</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td>Pigs</td>
<td>60</td>
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</tbody>
</table>

¹ MRL “not specified” means that available data on the identity and concentration of residues of the veterinary drug in animal tissues indicate a wide margin of safety for consumption of residues in food when the drug is used according to good practice in the use of veterinary drugs. For that reason, and for the reasons stated in the individual evaluation, the Committee concluded that the presence of drug residues in the named animal product does not present a health concern and that there is no need to specify a numerical MRL.
# World Health Organization Technical Report Series

**Recent reports:**

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<td>873</td>
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<td>1997</td>
<td>117</td>
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<td></td>
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<td>1998</td>
<td>97</td>
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<td>1998</td>
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<td>83</td>
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<td>1999</td>
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* Prices in developing countries are 70% of those listed here.