EVALUATION OF CERTAIN VETERINARY DRUG RESIDUES IN FOOD

Forty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives

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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. 41/9, 1997.

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**

The Joint FAO/WHO Expert Committee on Food Additives met in Rome from 4 to 13 June 1996. The meeting was opened by Mr J.R. Lupien, Director, Food and Nutrition Division, FAO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization.

Mr Lupien commended the members of the Committee for their hard work in expediting the assessment of residues of veterinary drugs in food by providing their valuable guidance and advice for the benefit of FAO, WHO, the World Trade Organization and other interested parties. He also expressed the appreciation of the two Organizations for the Committee’s efforts to make its assessments as clear as possible. At recent meetings, the Committee has had substances before it that are used both in animal health and as pesticides in plant production, and Mr Lupien emphasized the need for the collaboration of expert bodies working in closely related fields when such situations arise.

Eight 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 and 119) in response to the recommendations of the Joint FAO/WHO Expert Consultation held in 1984 (1). The present meeting¹ was convened in response to the recommendation made at the forty-fifth meeting of the Committee that meetings on this subject should be held annually (Annex 1, reference 119). 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 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);
- to determine criteria for appropriate methods of analysis for detecting or quantifying residues of veterinary drugs in food;

¹ As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives held in 1956 (FAO Nutrition Meeting Report Series, No. 11, 1956; WHO Technical Report Series, No. 107, 1956), there have been 46 previous meetings of the Joint FAO/WHO Expert Committee on Food Additives (Annex 1).
— to evaluate the safety of residues of certain veterinary drugs (see section 3 and Annex 2); and
— to discuss matters of interest arising from the report of the Ninth Session of the Codex Committee on Residues of Veterinary Drugs in Foods (2).

2. **General considerations**

2.1 **Principles governing the safety evaluation of residues of veterinary drugs in food**

In making recommendations on the safety of residues of veterinary drugs in food, the Committee took into consideration the principles contained in *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference 76) in the thirty-second, thirty-fourth, thirty-sixth, thirty-eighth, fortieth, forty-second, forty-third and forty-fifth reports of the Committee (Annex 1, references 80, 85, 91, 97, 104, 110, 113 and 119) and in the report of the Joint FAO/WHO Expert Consultation on Residues of Veterinary Drugs in Food (1).

2.2 **Modification of the agenda**

Abamectin and moxidectin were added to the agenda to further develop the evaluations initiated by the Committee at its forty-fifth meeting.

The evaluations of olaquindox, porcine somatotropin and spectinomycin were postponed to a future meeting at the request of the sponsors.

2.3 **Assessment of the effects of antimicrobial drug residues in food on the human intestinal microflora**

The microbiological risk associated with residues of antimicrobial agents in food resulting from their use in animals has been addressed previously at the thirty-second, thirty-sixth, forty-second and forty-fifth meetings of the Committee (Annex 1, references 80, 91, 110 and 119). At the present meeting, the Committee continued discussions on this issue based on a paper first considered at the forty-fifth meeting. Comments had been received on the draft paper, and these were considered in these discussions. The Committee’s views and conclusions are summarized in Annex 3.

The Committee reaffirmed its view that the possible adverse effects of residues of antimicrobial agents on human gastrointestinal microflora
should always be investigated. However, more research is needed to evaluate the potential health risks associated with the consumption of foods containing low levels of residues of antimicrobial agents, since such substances occur naturally in food of animal and plant origin.

A decision regarding microbiological safety should not normally be limited to one simple test system or to the use of data on the minimum inhibitory concentration (MIC) for deriving an ADI. When evaluating an antimicrobial agent, the Committee will normally require all available data on its microbiological activity and an evaluation report relating such data to possible effects on the human intestinal microflora. These investigations may be based on the results of studies using \textit{in vivo} or \textit{in vitro} models and/or other relevant data. The extent and nature of the data may differ, depending on the class of drug and on the extent to which it has been tested and/or used previously in humans and animals. Types of information useful to the Committee include the stability and bioavailability of the drug in the gastrointestinal tract, its spectrum of activity against different species of the gastrointestinal microflora, its effect on population changes in the intestinal microflora, its influence on the barrier to colonization \textit{in vitro} or \textit{in vivo}, and its potential to cause gastrointestinal disturbances in animals or humans. The Committee will consider all available relevant information when assessing a drug, which may include the use of the formula for the ADI developed at the thirty-eighth meeting (Annex 1, reference 97) and modified at the present meeting (see p. 79). The ADI may be based on either a toxicological or a microbiological end-point.

The Committee recognized the shortcomings of the methods currently used to evaluate the effects of antimicrobial drug residues in food on the human intestinal microflora. In addition, microbiological test systems designed to evaluate these effects have not been sufficiently validated to be fully satisfactory for this purpose. Therefore, in assessing the safety of antimicrobial agents, the Committee recommended that sponsors submit data on the safety evaluation of antimicrobial drugs for use in animals as follows:

1. In the absence of data from studies in humans, the microbiological ADI could be based on the results of studies on \textit{in vivo} models such as germ-free rodents colonized with human intestinal microflora ("human flora-associated" (HFA) rodents), or \textit{in vitro} models such as MIC data or continuous culture systems (see Annex 3, Table 3). HFA rodents have been used by several laboratories as an \textit{in vivo} model for determining the effects of therapeutic doses of antimicrobials. Although this model may have moderate
to high relevance in determining the effects of low levels of antimicrobials on human microflora, it still needs to be validated.

2. If MIC data from *in vitro* studies are to be used for assessing the safety of antimicrobial agents in the absence of data from *in vivo* studies, it is important to recognize the limitations of the microbiological assay. In addition, it is important to ensure that the microorganisms chosen for evaluation are representative of the human intestinal microflora (see Annex 3, Table 1) and that the MIC determinations are made under conditions that mimic those in the gastrointestinal tract. MIC<sub>50</sub> data expressed in μg/g<sup>1</sup> should be used in the equation developed at the thirty-eighth meeting of the Committee (Annex 1, reference 97) and modified at the present meeting (see Annex 3):

\[
\text{Upper limit of ADI (μg per kg of body weight)} = \frac{\text{MIC}_{50} (\mu g/g) \times \text{Mass of colonic contents (g)}}{\text{Fraction of oral dose} \times \text{Safety factor} \times \text{Body weight (kg)} \times \text{bioavailable}}
\]

where:

MIC<sub>50</sub> = Minimum concentration of an antimicrobial agent 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 this evaluation, the MIC<sub>50</sub> value is the mean MIC<sub>50</sub> for the strain(s) of the relevant species tested. Alternatively, the lowest MIC<sub>50</sub> value for the most sensitive species can be used.

A value of 220 g is used for the mass of the colonic contents and 60 kg is used to represent the average body weight of an adult (see Annex 3). The safety factor used to take account of uncertainty about the amount and relevance of MIC data available for review may range from 1 to 10. A value of 1 will be used when extensive relevant microbiological data are provided.

3. The Committee encouraged the development of better *in vitro* and *in vivo* methods that are relevant for determining the effects of low concentrations of antimicrobial agents on the human gastrointestinal microflora. While the Committee has on some occasions used a

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1 Although MIC<sub>50</sub> 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<sub>50</sub> value is converted to these units, it is assumed that the density of the experimental medium is 1 g/ml.
formula such as that given above for establishing ADIs based on in vitro data, it recognized that other in vivo and in vitro methods are under development, and solicited comments that may lead in the future to a better assessment of the potential human health risk of the dietary intake of residues of antimicrobial agents. As further information is received, the Committee may develop a procedure whereby no further data on the effects of antimicrobial residues on human intestinal microflora are required, provided that such residues in food do not exceed a threshold such as an average level of 1.5 mg per day, corresponding to 1 mg/kg in the diet (equivalent to 25 μg per kg of body weight per day).

2.4 Assessment of analytical methods
A major function of the Committee is to recommend MRLs and comment on the availability of suitable analytical methods capable of reliably measuring residues at the recommended MRLs in particular tissue matrices. The final responsibility for recommending suitable validated analytical methods to be recognized by the Codex Alimentarius for regulatory purposes rests with the Codex Committee on Residues of Veterinary Drugs in Food through its ad hoc Working Group on Methods of Sampling and Analysis.

For the Expert Committee to complete its evaluation, it must be assured that the sponsor has provided sufficient acceptable performance criteria for the analytical methods used to obtain residue-depletion data so that the reliability of these data can be assessed. The Expert Committee also reviews any information provided and comments on available methods which it considers to have the performance characteristics necessary to serve as regulatory methods once their reliability has been tested and established by interlaboratory studies at and below the MRL values assigned to various tissue matrices and species.

2.5 Deadlines for submission of data
When evaluating food additives at its forty-sixth meeting, the Committee noted that sponsors often do not submit data by the specified deadlines, making it difficult to produce adequately reviewed monographs in time for the meeting at which they are to be considered. The same is also true of veterinary drugs. At its present meeting, the Committee therefore extended the scope of the statement in its forty-sixth report (Annex 1, reference 122, section 2.2.3) that “data submitted after the specified deadline would normally be considered at a later meeting” to include veterinary drugs.
3. **Comments on residues of specific veterinary drugs**

The Committee considered for the first time two adrenoceptor agonists, two antimicrobial agents and two insecticides. It reconsidered two anthelmintic agents and five antimicrobial agents. The recommendations made with regard to these compounds, together with details of further information required, are given in Annex 2.

Toxicological monographs or monograph addenda were prepared on all the substances on which toxicological data were considered. Residues monographs or monograph addenda were prepared on all the substances.

3.1 **Adrenoceptor agonists**

3.1.1 **Clenbuterol**

Clenbuterol is a β-adrenoceptor agonist that exerts a potent broncholytic effect by acting preferentially on the β₂-adrenoceptors in smooth muscle, resulting in relaxation of the bronchi and a decrease in airway resistance. Similarly, as a result of selective binding to β₂-adrenoceptors in uterine smooth muscle cell membranes, relaxation of the uterus (tocolysis) occurs. Clenbuterol is manufactured as a 50:50 racemic mixture, most of its pharmacological activity being associated with the laevo form.

The drug, as the hydrochloride, is used for the treatment of respiratory disease in horses and cattle and as a tocolytic agent in cattle. Although not approved for use as a growth-enhancing or repartitioning agent, it is used for this purpose in farm species at doses many times those recommended for therapeutic treatment.

Clenbuterol has not previously been evaluated by the Committee.

*Toxicological data*

The Committee considered data on acute and short-term toxicity, reproductive toxicity, teratogenicity, genotoxicity and carcinogenicity. The results of studies on the pharmacokinetics and pharmacodynamics of clenbuterol in animals and humans were also considered.

Clenbuterol is well absorbed after oral administration to animals and humans; the drug is largely and rapidly excreted in the urine, with the majority of the remainder in the faeces (see p. 9).

Clenbuterol was moderately toxic in mice and rats after oral administration, with LD₅₀ values in the range 80–175 mg per kg of body weight. It was less toxic in the dog (LD₅₀ = 400–800 mg per kg of body weight).
weight. It was more toxic after parenteral administration, with LD_{50} values in the range 30–85 mg per kg of body weight after intravenous administration. The main signs of toxicity included lethargy, tachycardia and tonic–clonic convulsions.

The main effects noted in the repeat-dose studies were tachycardia and, at higher doses, necrosis of the myocardium. Such effects are common with β-agonist drugs. The necrosis of the myocardium was considered to be due to the reduction in myocardial perfusion which resulted from the tachycardia.

In 30-day repeat-dose studies in mice and rats, no-observed-effect levels (NOELs) of 2.5 and 1 mg per kg of body weight per day, respectively, were identified, in most cases based on cardiac lesions. However, in a range of repeat-dose studies in rats given doses of 0.01–100 mg per kg of body weight per day by the oral, intravenous and inhalation routes for up to 18 months, no NOELs were identified. Effects were mostly related to cardiac function and were seen even at the lowest doses used. Similarly, no NOELs could be identified in a range of repeat-dose studies in dogs in which oral doses ranging from 0.1 to 40 mg per kg of body weight per day were used. In a 26-week study in cynomolgus monkeys (Macaca fascicularis) given the drug by inhalation, the NOEL was 25 μg per kg of body weight per day, based on cardiac and other effects.

No evidence of carcinogenicity was noted in a 2-year oral study in mice with doses of up to 25 mg per kg of body weight per day. Similarly, no evidence of carcinogenicity was seen in a 2-year study in Chbb: THOM rats given doses of up to 25 mg per kg of body weight per day. However, in Sprague–Dawley rats given 25 mg of clenbuterol per kg of body weight per day orally for 2 years, an increased incidence of mesovarian leiomyomas occurred. With the related compounds salbutamol in rats and medroxalol in mice, the effects could be abolished by administration of the β-adrenoceptor blocking agent propranolol. Mesovarian leiomyomas in rats and uterine leiomyomas in mice are known to occur following long-term treatment with β-adrenoceptor agonists, and the Committee concluded that these tumours were due to adrenergic stimulation and not to any genotoxic mechanism. Clenbuterol was not genotoxic in a range of in vitro and in vivo genotoxicity studies.

Epidemiological studies indicate that there has been no increase in the incidence of uterine leiomyomas in women following the use of β-adrenoceptor agonists.

Clenbuterol had no effects on fertility in a reproductive toxicity study in rats using oral doses of 1–50 mg per kg of body weight per day from
10 weeks before mating in males and 2 weeks before mating in females until termination of the study. However, all the offspring born to animals in the highest-dose group either were stillborn or died shortly after birth. To investigate the cause of the high pup mortality at the highest-dose level, the litters of control dams were exchanged with those from dams given 50 mg per kg of body weight per day. Pups from rats given the highest dose died on the first day of lactation, regardless of whether they suckled on treated or control dams. The mechanism involved in this lethal effect is unknown. A NOEL was not identified in this study because pup weights at birth were reduced in all treated animals.

In a reproductive toxicity study in which male rats were treated orally for 70 days prior to mating and females for 14 days prior to mating with 1.5–15 μg of clenbuterol per kg of body weight per day, no adverse effects on reproduction were noted. The NOEL was 15 μg per kg of body weight per day.

In teratogenicity studies in rats, oral doses of 10 and 100 mg per kg of body weight per day produced teratogenic effects that included hydrocephalus, anasarca, umbilical hernia, anophthalmia, rib variations and splintering of vertebrae. These were accompanied by signs of maternal toxicity. The NOEL was 1 mg per kg of body weight per day. In three studies in rabbits using doses ranging from 30 μg per kg of body weight per day to 50 mg per kg of body weight per day, signs of fetotoxicity, including delayed ossification and cleft palate, were seen. The NOEL was 30 μg per kg of body weight per day.

Clenbuterol produced a range of pharmacodynamic effects, including tachycardia, hypertension and muscle-relaxing effects, in a number of animal species. These were seen at single doses as low as 0.8 μg per kg of body weight.

Four metabolites of clenbuterol that had been shown to be present in the kidneys of treated target animals were tested for pharmacological activity. Of these, only one was shown to have activity. Its broncholytic effect in the guinea-pig was less than 20% of that of clenbuterol and, in addition, it accounted for only 1–2% of residues in liver and kidney of target animals 6 hours after treatment.

In humans, clenbuterol produced a broncholytic effect when a single dose of 10 μg (equivalent to 0.167 μg per kg of body weight) was given by inhalation, but no evidence of tachycardia was seen at this dose. With oral doses of up to 5 μg per day (equivalent to 0.08 μg per kg of body weight per day) for 3 days there were no effects on bronchial resistance, thoracic gas volume, cardiac rate or blood pressure. The
NOEL in this study was 0.08µg per kg of body weight per day. In a study to investigate the bronchospasmolytic effect in humans, patients with obstructive lung disease were given single oral doses of up to 30µg per person. Patients administered doses of 5µg or above exhibited bronchospasmolytic effects. The NOEL in this study was 2.5µg per person, equivalent to 0.04µg per kg of body weight.

The Committee considered the most relevant study for determining the ADI to be that on the bronchospasmolytic effect in humans. The patients had chronic obstructive airway disease and thus were likely to be a very sensitive population for this effect. The NOEL identified in this study (2.5µg per person, equivalent to 0.04µg per kg of body weight) is approximately 25% of the dose in another study in which the inhalation route was used, but in which cardiac effects were not observed. Similarly, this NOEL is approximately 50% of the oral dose used in another study where, again, cardiac effects did not occur. Hence, this NOEL for the bronchospasmolytic effect offers an additional safety margin for cardiac effects. The Committee therefore established an ADI of 0–0.004µg per kg of body weight, based on the NOEL of 0.04µg per kg of body weight for pharmacodynamic effects in humans and a safety factor of 10.

Pharmacokinetic data
Clenbuterol was well absorbed after oral administration to laboratory animals, humans and the target species. In most species peak blood concentrations were achieved 2–3 hours after oral dosing. The plasma half-life in cattle varied from 16 to 105 hours. The substance was widely distributed in the tissues and was shown to cross the placenta in pregnant rats, dogs, baboons and cows. In all species, excretion was predominantly via the urine as unmetabolized clenbuterol.

Metabolic data
Eight studies on cattle given [¹⁴C]clenbuterol either orally or as an intramuscular or intravenous injection showed that most (50–85%) of the dose was excreted in the urine within 4–15 days after dosing, with lesser amounts excreted in the faeces (5–30%) and, in lactating cattle, in milk (0.9–3%). After oral administration of the radiolabelled drug to horses, 75–91% and 6–15% of the dose was excreted in the urine and faeces, respectively, over a 14-day period. The metabolic pathways were similar in all the species studied, though there were quantitative differences in the amounts of metabolites formed.

The metabolite profile seen in cattle was qualitatively similar to that seen in laboratory animals and humans. The metabolism of radiolabelled clenbuterol in bovine liver followed a similar pattern, the
majority of the extractable residues (>50%) being clenbuterol. There were four minor metabolites and some unidentified polar metabolites.

Investigations in horses given [\textsuperscript{14}C]clenbuterol revealed that the parent compound accounted for 38–90% of total radioactivity in the liver at 12 and 24 hours after dosing. Apart from clenbuterol, one metabolite formed by the hydroxylation of a tertiary methyl group (NA 1141) (10%) and NAB 821 (R–CHOH–CH₂OH) (3–7%) could be identified in equine liver tissue. After 24 hours there was a quantitative change in the pattern of metabolites seen, though not a qualitative one. The metabolism of clenbuterol in horse kidney is similar to that described in the liver. The parent compound accounted for 89% of the extracted radioactivity in the kidney at 24 hours after treatment, while NA 1141 accounted for the remaining 11%.

Residue data
All the residue studies submitted by the sponsor were carried out using the \textsuperscript{14}C-radiolabelled racemic (chiral) mixture and satisfied the requirements of good laboratory practice.

In cattle the total residues were much higher in animals treated with multiple doses of 0.8 µg per kg of body weight twice daily for 5.5–10.5 days than in those given a single injection. In both types of treatment the highest levels of residues were observed in liver and kidney, while very low levels were present in muscle and fat. Total residues were <0.3 µg/kg in muscle and fat from about 6 days after treatment with multiple doses, but between 0.35 and 9.85 µg/kg in liver and kidney at 6–28 days after dosing. Residues in muscle and fat consisted mostly of clenbuterol shortly after administration, but in liver and kidney the percentage declined with increasing withdrawal time. The relationship between residues of clenbuterol and total residues was determined at 6 hours, 3 days and 6 days after treatment. Residues in muscle (including the injection site) consisted mostly of clenbuterol. At 6 hours after treatment, the parent compound accounted for almost all the residues in liver, but after 6 days the percentage had declined to less than 50%.

Residues at the injection site in muscle varied and there was no correlation between the concentration range and time during the first 11 days after dosing. However, in one study the residues were low (<0.25 µg/kg) at 28 days after dosing.

Use of the drug as a tocolytic agent may result in residues in milk in the period following parturition. Potential levels of residues in milk as a result of this treatment were investigated by giving lactating cows a
single intramuscular injection of 0.8μg per kg of body weight of \[^{13}C\]clenbuterol. The residues in milk over a 3-day sampling period consisted almost entirely of unmetabolized clenbuterol. The very low concentrations of radioactivity (0.015–0.050μg/l at 79 hours after injection) at subsequent times did not appear to be clenbuterol. To determine whether the drug could be administered to lactating cows in its multiple-dose formulation, three cows were given intramuscular injections of 0.8μg per kg of body weight of \[^{13}C\]clenbuterol twice daily for 3 days, followed by twice daily oral doses for 2 days and a single oral dose on day 6. Total residues in milk reached peak values of 3.2, 3.5 and 3.9μg/l during administration, but had declined to 0.18μg/l by 108 hours after dosing in the one cow kept in the study. Because of these unacceptably high levels of residues, this formulation is not recommended for use in lactating cattle.

Two studies were carried out in horses in which the concentrations of total residues in tissues were compared with residues of unmetabolized clenbuterol. In both studies the pattern of residue depletion was similar to that in cattle. Three horses were given oral doses of 0.8μg per kg of body weight twice daily for 10 days, followed by a single oral dose on day 11. At 6 days after withdrawal the residue levels were all less than 0.3μg/kg in muscle, kidney and fat but greater than 3μg/kg in liver. The same dosing regimen was used in a second study, in which 12 ponies were dosed with the radiolabelled drug and total residues were measured at 0.5, 3, 12 and 28 days after dosing. At all times the total residues were <1μg/kg in liver, <0.2μg/kg in kidney, and <0.1μg/kg in muscle and fat.

**Analytical methods**

More than 100 methods for the determination of residues of clenbuterol and other similar β-adrenoceptor agonists in biological samples have been published in the literature since 1990. The methods used for screening include enzyme immunoassay, high-performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC–MS). Positive samples are confirmed by means of specific GC–MS methods with detection limits for edible tissues of ≥0.01μg/kg and quantification limits of ≥0.02μg/kg. The manufacturer’s proposed method for routine analysis of samples was based on GC–MS. The quantification limit was stated to be 0.10μg/kg for tissues and 0.05μg/l for milk, but acceptable accuracy and precision had not been demonstrated at these concentrations. Another well validated method presented by the manufacturer, also based on GC–MS, had been shown to have a quantification limit of 0.020μg/kg and a detection limit of 0.010μg/kg for bovine tissues.
Maximum Residue Limits
In reaching its decision on MRLs for clenbuterol, the Committee took the following factors into account:

- The ADI of 0–0.004 μg per kg of body weight per day, which is equivalent to a maximum ADI of 0.24 μg for a 60-kg person.
- Muscle and liver are the target tissues.
- The parent drug is the marker residue and is the only residue of public health concern. It accounts for 100% of the total residues in muscle, fat and milk (cows’), 60% of the total residues in bovine liver and kidney, and 6% of the total residues in equine liver and kidney. Because the metabolites and bound residues are not of toxicological concern, they do not need to be taken into account in the calculation of the MRLs.
- Analytical methods suitable for regulatory use are available.
- The sponsor is not proposing to make the drug available for use in lactating cows.

The Committee recommended MRLs in cattle and horses of 0.2 μg/kg for muscle and fat, 0.6 μg/kg for liver and kidney, and 0.05 μg/l for milk (cows’), expressed as parent drug. These MRLs would result in a maximum daily intake of 0.235 μg, 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 (Annex 1, reference 85).

The Committee was aware that clenbuterol has been used illegally as a growth-enhancing agent, primarily in cattle. When used for this purpose, it must be administered up to the time of slaughter. Residue studies conducted under these conditions are available, and both these and results of regulatory monitoring indicate that residues are likely to be substantially in excess of the MRLs, particularly in liver. The Committee recommended that clenbuterol should not be used as a growth-enhancing agent.

3.1.2 Xylazine
Xylazine is a clonidine analogue which acts on presynaptic and postsynaptic receptors of the central and peripheral nervous systems. It is an α2-adrenoceptor agonist used in animals for its tranquillizing, muscle relaxant and analgesic effects, but has numerous other pharmacological effects, the most important being bradycardia and hypotension. Xylazine inhibits the effects of postganglionic cholinergic nerve stimulation.

Xylazine had not been previously evaluated by the Committee.
Toxicological data

A range of studies on xylazine were available for evaluation by the Committee, including data on pharmacodynamics, pharmacokinetics, acute and short-term toxicity, reproductive and developmental toxicity, genotoxicity and effects in humans. The Committee also reviewed data from toxicological studies on 2,6-xylidine, a metabolite of xylazine, including the results of studies on its acute and short-term toxicity, carcinogenicity and genotoxicity.

Numerous pharmacological side-effects of xylazine have been observed in treated animals, including mydriasis, impairment of thermoregulatory control, various effects on the acid-base balance and respiration, hyperglycaemia, and cardiovascular, haematological and gastrointestinal effects. Cattle and sheep are approximately 10 times as sensitive to xylazine as horses, dogs and cats.

Pharmacokinetic data on the parent compound from studies in cattle, horses, sheep, dogs and laboratory animals were reported (see p. 15).

In acute oral toxicity studies in mice and rats, xylazine was found to be moderately toxic, with LD$_{50}$ values of the order of 121–240 mg per kg of body weight. 2,6-Xylidine was found to be slightly toxic, with LD$_{50}$ values of the order of 600–1000 mg per kg of body weight.

Three studies on the short-term toxicity of xylazine were reviewed. A 32-week dietary study in rats and a 16-week study in dogs given xylazine capsules were considered inadequate for the determination of the toxicity of xylazine owing to the insufficient numbers and poor quality of the animals used and the inadequacy of the study designs. In the third study, beagle dogs were fed xylazine in the diet at 10, 30 or 100 mg/kg (equal to 0.3, 0.9 or 3 mg per kg of body weight per day) for 13 weeks. No treatment-related effects were observed in any of the treated groups.

Two studies on the short-term toxicity of 2,6-xylidine were reviewed. In the first study, rats were dosed with 80, 160, 310, 620 or 1250 mg per kg of body weight per day by gavage on 5 days per week for 2 weeks. Treatment-related effects included increased mortality (all 10 animals in the highest-dose groups died), decreased body weight (at 310 mg per kg of body weight per day and above in males and 160 mg per kg of body weight per day and above in females), and various effects on haematological parameters as indicated by leukocytosis and changes in red blood cell parameters indicative of increased erythropoiesis (at 310 mg per kg of body weight per day and above in both sexes). The NOEL in this study was 80 mg per kg of body weight per day.
In the second short-term toxicity study of 2,6-xylidine, rats were
dosed with 20, 40, 80, 160 or 310mg of 2,6-xylidine per kg of body
weight per day by gavage on 5 days per week for 13 weeks.
Treatment-related effects included decreased body-weight gain (in
males at 310mg per kg of body weight per day and in females at 40mg
per kg of body weight per day and above), increased absolute and
relative liver weights (in males at 310mg per kg of body weight per
day and in females at 160mg per kg of body weight per day and
above), leukopenia (in males at 40mg per kg of body weight per day
and above), haemoglobinemia (in males at 160mg per kg of body
weight per day and above) and anaemia (in males at 310mg per kg of
body weight per day). The NOEL in this study was 20mg per kg of
body weight per day.

In a carcinogenicity study, male and female rats were fed diets con-
taining 2,6-xylidine at concentrations of 300, 1000 or 3000mg/kg
(equivalent to 15, 50 or 150mg per kg of body weight per day).
Significant increases in the incidences of papillomas and carcinomas
of the nasal cavity were observed in both males and females given the
highest dose. There was a significant dose-related increase in the
incidence of adenomas in the nasal cavity in both sexes. In addition,
rhabdomyosarcomas and malignant mixed tumours of the nasal cavity
were observed in animals in the highest-dose group. There was a
significant dose-related increase in the incidence of subcutaneous
fibromas and fibrosarcomas in both sexes. In females, there was a
significant positive trend in the incidence of neoplastic nodules in the
liver. The Committee concluded that 2,6-xylidine was carcinogenic in
this study.

The International Agency for Research on Cancer has evaluated the
carcinogenic risk of 2,6-xylidine and has classified it in Group 2B
(possibly carcinogenic to humans) (3).

In a teratogenicity study, xylazine was administered to pregnant rats
at doses of 1, 4 or 16mg per kg of body weight per day on days 6–15
of gestation. Treatment-related maternal effects included partial
closing of the eyelids, hypoactivity, ataxia, flat posture and slightly
reduced body-weight gain in the highest-dose group. A decrease in
mean fetal weight was seen in the highest-dose group. No teratogenic
effects were noted in this study. The NOEL for maternal and fetal
effects was 4mg per kg of body weight per day.

Xylazine has been tested in reverse mutation assays in Salmonella, a
forward mutation assay in cultured mammalian cells, and an in vivo
cytogenetic assay. In Salmonella, weak positive results were obtained.
Negative results were observed in the forward mutation assay on
cultured mammalian cells and in a micronucleus test in mouse bone marrow. The Committee concluded that xylazine was weakly mutagenic.

2,6-Xyldine was tested in a series of in vitro and in vivo genotoxic assays. It was weakly positive for reverse mutation in Salmonella. In mammalian cells, it induced forward mutation and was positive in a sister chromatid exchange test. Inconclusive results were obtained in a micronucleus test in mouse bone marrow because there was no assurance that the bone marrow had been adequately exposed. 2,6-Xyldine was found to be inactive in an in vivo-in vitro assay for unscheduled DNA synthesis in rat hepatocytes. Covalent binding of the compound to DNA was observed in rats. The Committee concluded that 2,6-xyldine is genotoxic.

The potential for 2,6-xyldine to induce methaemoglobinaemia was reviewed by the Committee. Single doses of 30mg of 2,6-xyldine per kg of body weight intravenously or 164mg of N-acetyl 2,6-xyldine per kg of body weight orally have been shown to induce met-haemoglobinaemia in cats, but not in dogs. 2,6-Xyldine has also been detected in humans given lidocaine. Methaemoglobin and 2,6-xyldine–haemoglobin adduct levels have been shown to increase in patients with cardiac disease following treatment with lidocaine.

Effects of xylazine on humans poisoned following accidental or intentional self-injection (0.7–15mg per kg of body weight) or ingestion (7mg per kg of body weight) included symptoms of central nervous system depression, respiratory depression, hypo- and hypertension, bradycardia, tachycardia, ventricular arrhythmias and transient hyperglycaemia.

The Committee was unable to establish an ADI for xylazine because it concluded that the metabolite 2,6-xyldine was genotoxic and carcinogenic.

Pharmacokinetic data
Rats were given radiolabelled xylazine intravenously at doses of 0.02–10mg per kg of body weight or orally at doses of 0.02–100mg per kg of body weight. More than 95% of the oral dose was absorbed, with a half-life of approximately 5 minutes. Following oral or intravenous administration, approximately 70% of the dose was eliminated in the urine with a half-life of 2–3 hours. The remainder was eliminated in the faeces. Enterohepatic circulation did not occur to a notable extent. In cattle given an intramuscular dose of 0.2 or 0.5mg of xylazine per kg of body weight, less than 1% of the dose was excreted unchanged in the urine, and the parent compound was detected in the
urine for up to 6 hours following administration. Metabolites of xylazine were detected in urine from these cattle for up to 10 hours after treatment.

Pharmacokinetic parameters following intravenous administration showed minor variations between species. Xylazine was eliminated rapidly from plasma following intravenous administration, with a half-life of approximately 40 minutes in cattle and 20 minutes in sheep. Xylazine could not be detected in plasma of cattle following intramuscular administration of a single therapeutic dose.

Species differences in elimination indicate differences in metabolic activity and/or different metabolic pathways. The apparent volume of distribution was large (1.9–2.5 litres/kg) due to the lipophilic nature of the drug. The depletion of the unlabelled compound from plasma in cattle was more rapid than the depletion of total radioactivity in a similar study using [14C]xylazine. Therefore, clarification of xylazine metabolism is required in order to better understand its pharmacokinetics.

*Metabolism data*

In rats given an intravenous dose of 2 mg per kg of body weight of radiolabelled xylazine, approximately 20 metabolites were quantified as xylazine equivalents in urine and bile. The major metabolite accounted for 35% of the administered dose. Approximately 8% of the dose was eliminated as unchanged xylazine 24 hours after dosing. In an *in vitro* study, four metabolites were identified when xylazine was incubated with rat liver microsomes. The same metabolites were identified in the urine of horses treated with an intravenous dose of xylazine. The major metabolite in both cases was identified as N-(2,6-dimethylphenyl)thiourea. In cattle given an intramuscular dose of 0.2 mg of xylazine per kg of body weight (two cows) or 0.5 mg of xylazine per kg of body weight (one cow), 2,6-xyldine was identified in urine in both conjugated and unconjugated forms.

However, no data on xylazine metabolism in other animals were available. For this reason, the possibility that metabolism causes the discrepancy between the residue-depletion studies using the radiolabelled compound and those using the unlabelled compound could not be evaluated.

*Residue data*

Residue-depletion studies were conducted in cattle (three calves and one lactating cow), which were slaughtered at different time intervals after intramuscular administration of 0.33 mg per kg of body weight of [14C]xylazine labelled in the thiazine ring. The excretion of the
radioactivity was complete by 74 hours after administration. Between 63% and 77% of the radioactivity was excreted in the urine and 3–23% in the faeces. In a related study in three calves and two lactating cows, which received 0.33 mg per kg of body weight of [14C]xylazine labelled in the aniline ring by the intramuscular route, the excretion of the radioactivity was variable, ranging from 38% to 99%. In the second study, the ratio of the amount of radioactivity excreted in the urine to that excreted in faeces ranged from 12:1 to 6:1.

In the two residue-depletion studies reported above, the total residue levels in kidney, liver and injection-site tissue were 0.009–0.020, 0.022–0.050 and 0.030–1.152 mg/kg xylazine equivalents, respectively, 72 hours after administration, regardless of the site of the radiolabel in the molecule. In contrast, the radioactivity in milk declined to 0.01 mg/l 60 hours after treatment with [14C]xylazine labelled in the thiazine ring and 12 hours after treatment with [14C]xylazine labelled in the aniline ring.

The data obtained from residue-depletion studies in cattle using unlabelled xylazine in which only the concentration of the parent drug was determined were in clear contrast to those from the studies using the radiolabelled drug. In the studies using the unlabelled drug, residues were below the limit of detection (0.01 mg/kg) of the analytical method in muscle, kidney, liver or fat, while residues in milk were above the limit of detection only temporarily. Thus, most of the residues were not parent drug, but unidentified metabolites.

Analytical methods
A number of analytical methods, mainly for the parent drug, were described, including photometry, HPLC and GC-MS. Their performance characteristics were poorly determined but a quantification limit of 0.01 mg/kg was claimed. No validation data for the methods were available for evaluation.

Maximum Residue Limits
The Committee did not recommend MRLs for xylazine because:

— no ADI was established;
— the data on the metabolism of the compound were inadequate;
— the residue data available to the Committee were not sufficient for it to identify a marker residue;
— the residue-depletion studies were inadequate.

The Committee requires the following information before reviewing this compound further:
• Sufficient data on the metabolism of xylazine in target species for it to identify a suitable marker residue and target tissue(s).
• A suitable analytical method for measuring the marker residue in the target tissue(s).
• Additional data on the depletion of residues of xylazine and its metabolites in target species, including evidence to show whether 2,6-xylidine is present at the recommended withdrawal times.

3.2 Anthelmintic agents

3.2.1 Abamectin

Abamectin is used both as a pesticide and as an anthelmintic drug in animals. It has been evaluated toxicologically by two Joint FAO/WHO Meetings on Pesticide Residues, held in 1992 and 1994 (4, 5). At the latter Meeting, an ADI of 0–0.2 µg per kg of body weight was established, based on a no-observed-adverse-effect level of 0.12 mg per kg of body weight per day for pup toxicity in a reproductive toxicity study in rats. A safety factor of 500 was applied because of concern about the teratogenicity of the Δ-8,9 isomer, a photolysis product that has been detected as a residue in plant products.

The use of abamectin as a veterinary drug was considered at the forty-fifth meeting of the Committee (Annex 1, reference 119). The Committee intended to rely on the toxicological evaluation performed by the 1994 Joint FAO/WHO Meeting on Pesticide Residues, but on reviewing the data relating to the use(s) of abamectin, concluded that the Δ-8,9 isomer is not present in animal tissues when abamectin is used as a veterinary drug. Therefore, the Committee recommended that consultations be held as soon as possible between representatives of the Committee and the Joint FAO/WHO Meeting on Pesticide Residues. Such a meeting was held in September 1995, at which time it was recognized that consideration should be given to establishing different ADIs for abamectin as a pesticide and as a veterinary drug. As a consequence, the 1995 Joint FAO/WHO Meeting on Pesticide Residues (6) agreed that the ADI of 0–0.2 µg per kg of body weight was not appropriate for abamectin residues that do not contain the Δ-8,9 isomer. In order to accommodate this situation, the Joint FAO/WHO Meeting on Pesticide Residues allocated an ADI of 0–1 µg per kg of body weight to abamectin, based on the NOEL of 0.12 mg per kg of body weight per day in the study on reproductive toxicity in rats and a safety factor of 100.

Residue data

The 1995 Joint FAO/WHO Meeting on Pesticide Residues (6) emphasized that the MRLs recommended for abamectin should
include residues resulting from the use of abamectin as a veterinary drug as well as from the consumption by animals of fodder containing residues of abamectin.

The MRLs recommended by the 1992 Joint FAO/WHO Meeting on Pesticide Residues (4) for abamectin in cattle were: 0.01 mg/kg for muscle, and 0.05 mg/kg for liver and kidney.

In considering its decision on the MRLs for abamectin, the Committee took the following factors into account:

- The ADI of 0–1 µg per kg of body weight, which was established by the 1995 Joint FAO/WHO Meeting on Pesticide Residues (6). This would result in a maximum daily intake of residues of 60 µg for a 60-kg person.
- Abamectin used as a veterinary drug is intended only for use in beef cattle.
- Avermectin B₁₉ is considered to be the appropriate marker residue.
- Liver and fat are considered to be the appropriate target tissues.
- No bound residues are formed in fat tissue and such residues account for less than 15% of total residues in liver.
- Avermectin B₁₉ accounts for 42%, 50% and 25% of the total residues in liver, kidney and fat, respectively, at 21 days after dosing.
- A validated analytical method is available.

The Committee recommended the following MRLs for cattle which, for abamectin used as a veterinary drug, are expressed as avermectin B₁₉:

- Fat and liver — 100 µg/kg
- Kidney — 50 µg/kg.

The Committee noted that there was no need for an MRL for cows’ milk, since abamectin is intended only for use in beef cattle. There was also no need for an MRL for bovine muscle, since abamectin residues are below detectable levels at the recommended withdrawal time. Liver, kidney and fat were the appropriate tissues for monitoring residues of abamectin in animal tissues. Nevertheless, the Committee recognized that the 1992 Joint FAO/WHO Meeting on Pesticide Residues (4) had established MRLs for abamectin in cattle muscle and milk resulting from pesticide use.

The MRLs recommended by the Committee result in a theoretical maximum daily intake of total residues of abamectin of 49 µg (see Table 1). Since the maximum ADI is 60 µg, this gives an acceptable margin of safety for the possible additional ingestion of residues from
Table 1
Theoretical maximum intake of abamectin 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 abamectin equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>100</td>
<td>240(^a)</td>
<td>24</td>
</tr>
<tr>
<td>Kidney</td>
<td>50</td>
<td>100(^a)</td>
<td>5</td>
</tr>
<tr>
<td>Fat</td>
<td>100</td>
<td>400(^a)</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>49</td>
</tr>
</tbody>
</table>

\(^a\) Expressed as avermectin B₁.

\(^b\) Based on a daily intake of 100 g of liver, 50 g of kidney and 50 g of fat.

pesticide use resulting from the consumption both of fruits and vegetables and of meat from cattle ingesting contaminated fodder.

3.2.2 Moxidectin

Moxidectin was first evaluated at the forty-fifth meeting of the Committee (Annex 1, reference 119), when an ADI of 0–2 µg per kg of body weight was established. MRLs or temporary MRLs were recommended in cattle, sheep and deer.

Residue data

At its forty-fifth meeting, the Committee recommended the following MRLs for cattle, sheep and deer: 20 µg/kg for muscle, 100 µg/kg for liver, 50 µg/kg for kidney and 500 µg/kg for fat, expressed as parent drug. For deer, the MRLs were temporary.

At its present meeting, the Committee evaluated a new residue-depletion study in sheep, which were treated according to the recommended dosing schedule for psoroptic mange (0.2 mg of moxidectin per kg of body weight as a subcutaneous injection, repeated after 10 days). In this study five groups of six lambs (three females and three castrated males) were treated with moxidectin, 1.0% injectable solution; 9 animals served as controls or as replacements. All of the animals in the first treatment group and two of the untreated controls were killed 10 days after treatment, and samples of back fat, loin muscle, kidney, liver and injection site muscle were removed and frozen for residue analysis. At the same time, all the animals in the remaining treatment groups were given a second injection of moxidectin on the opposite side of the neck. The animals in these groups were killed at 20, 30, 40 and 50 days after treatment, respectively, and tissue samples were removed and analysed for residues of moxidectin.
Three main points emerged from the study:

1. The residue levels in muscle consistently exceeded the MRL proposed at the forty-fifth meeting of the Committee. The highest levels (63µg/kg) occurred 10 days after dosing, but thereafter (20–50 days) the levels did not exceed 40µg/kg, even though two doses were administered.

2. The residue levels in liver, kidney and fat did not exceed the recommended MRLs.

3. The residue levels at the injection sites were very high and persistent.

In the submission reviewed at the forty-fifth meeting, residues of moxidectin were not measurable (<10µg/kg) in the muscle of sheep by 28 days. However, in the new study it was clear that residues in ovine muscle persisted for at least 50 days. The Committee suggested that the presence of measurable levels of residues in ovine muscle but not in bovine or deer muscle is probably due to the higher fat content of sheep muscle and the lipophilic nature of moxidectin.

Maximum Residue Limits

The ADI established at the forty-fifth meeting of the Committee was 0–2µg per kg of body weight, which is equivalent to a maximum ADI of 120µg for a 60-kg person. The MRLs proposed at that time resulted in a maximum theoretical intake of 79µg per day. In view of the observed residues in sheep muscle in the new study, the Committee recommended that the MRL for sheep muscle be increased to 50µg/kg. Since moxidectin accounts for 40% of the total residues in muscle, this would result in an increase from 15 to 38µg of moxidectin equivalents for sheep muscle (see Table 2). The ADI would not be exceeded

<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 moxidectin equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>50</td>
<td>125º</td>
<td>38</td>
</tr>
<tr>
<td>Liver</td>
<td>100</td>
<td>250º</td>
<td>25</td>
</tr>
<tr>
<td>Kidney</td>
<td>50</td>
<td>125º</td>
<td>6</td>
</tr>
<tr>
<td>Fat</td>
<td>500</td>
<td>665º</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>102</td>
</tr>
</tbody>
</table>

* Expressed as parent drug.
* Based on daily intake of 0.5 kg of meat made up of 300 g of muscle, 100 g of liver, 50 g of kidney and 50 g of fat.
* The marker residue accounted for 40% of the total residues in muscle, liver and kidney.
* The marker residue accounted for 78% of the total residues in fat.
with this new MRL. The Committee confirmed all the other MRLs recommended at the forty-fifth meeting.

3.3 **Antimicrobial agents**

3.3.1 **Chlortetracycline, oxytetracycline and tetracycline**

An ADI of 0–3 μg per kg of body weight was established for oxytetracycline at the thirty-sixth meeting of the Committee, based on a microbiological end-point (Annex 1, reference 91). MRLs of 100 μg/kg in muscle, 300 μg/kg in liver, 600 μg/kg in kidney, 10 μg/kg in fat and 200 μg/kg in eggs were recommended, and the MRL of 100 μg/l in milk, recommended at the twelfth meeting (Annex 1, reference 17), was retained.

At its forty-fifth meeting (Annex 1, reference 119), the Committee established a group ADI of 0–3 μg per kg of body weight for chlortetracycline, oxytetracycline and tetracycline, alone or in combination. However, the Committee noted that the MRLs allocated to oxytetracycline at the thirty-sixth meeting (Annex 1, reference 91) resulted in the ADI being exceeded when the standard daily food intake (Annex 1, reference 85) was used to calculate the theoretical maximum daily intake. The major contributory factor to this situation was the MRL of 100 μg/l allocated to milk.

At its forty-fifth meeting, the Committee therefore requested the following information for evaluation in 1996:

1. The results of residue-depletion studies in cattle, sheep, pigs and poultry treated with chlortetracycline and tetracycline in accordance with approved uses, to determine the depletion of residues in milk (cattle), fat (all species) and muscle, liver and kidney (sheep).
2. New and validated methods of analysis for chlortetracycline, oxytetracycline and tetracycline residues in milk.

Pending the outcome of this evaluation, temporary MRLs were recommended for both chlortetracycline and tetracycline in cattle, pigs and poultry of 100 μg/kg for muscle, 300 μg/kg for liver, 600 μg/kg for kidney and 200 μg/kg for eggs (poultry), expressed as parent drug. Temporary MRLs of 300 μg/kg for liver and 600 μg/kg for kidney of sheep were also recommended, expressed as parent drug.

*Residue data*

A detailed comparison of chlortetracycline levels in fat and kidney of cattle, pigs, sheep and poultry at various times after withdrawal of medication indicated that residue levels in fat were one-ninth or less of those found in kidney and depleted far more rapidly. Cattle given
chlortetracycline in the feed at 22 mg per kg of body weight per day for 10 days had mean residue levels in fat of 40 µg/kg 7 days after dosing, whereas mean levels in kidney, liver and muscle were 450, 270 and 140 µg/kg, respectively. Pigs given chlortetracycline in the diet at 400 mg per kg of body weight per day for 7 days had mean residue levels of 100 µg/kg in fat at the time of withdrawal of the drug and mean residue levels of 2690 and 1320 µg/kg in kidney and liver, respectively. Two separate studies in chickens given chlortetracycline at 220 mg/kg in the feed showed residue levels of 660 and 710 µg/kg in liver and 420 and 750 µg/kg in kidney, respectively, at the time of withdrawal of medication compared to levels of 20 and 40 µg/kg in skin with adhering fat. No residues of chlortetracycline were detected in fat 1 day after withdrawal of the drug. Another study found no chlortetracycline residues in fat of chickens given medicated diets containing 110 mg/kg of chlortetracycline with 121 mg/kg of monensin for up to 51 days, 1 day after withdrawal of medication.

Because tetracyclines are rapidly depleted in fat, the Committee concluded that fat is not an appropriate target tissue for this class of drug and that an MRL for fat is not required.

Analytical methods
Recent HPLC methods of tetracycline analysis individually identify and quantify all three tetracyclines at levels at or well below the MRLs allocated to them. Two validation trials have been published in 1996, and these clearly show detection and quantification levels in all tissues that allow regulation of assigned MRLs. Furthermore, a published validation study in milk demonstrates that current methodology would readily permit a lowering of the MRL of tetracyclines in milk to 50 µg/l. This validated quantitative method is also supported by both microbiological and immunochemical screening methods with the requisite detection levels and performance characteristics.

Notwithstanding the capability of analytical methods to identify and quantify residues at a lower MRL in milk, the Committee decided to retain the MRL of 100 µg/l for oxytetracycline in milk and recommended the same MRL for chlortetracycline and tetracycline. In maintaining this MRL, the Committee considered data showing that, for oxytetracycline, levels in milk fell below 100 µg/l only after 6–8 milkings following intramammary infusion or 10–14 milkings following administration of long-acting formulations for intramammary infusion. Data for chlortetracycline also show that at least 6–10 milkings would be necessary following administration of formulations for intramammary infusion to ensure that levels in milk were below
100μg/l. A lowering of the MRL to 50μg/l would result in unacceptable withdrawal times for milk.

The Committee also reaffirmed the opinion expressed at the thirty-sixth meeting that no risk to human health would result if the ADI of 180μg were exceeded by 30% if the MRLs previously established for oxytetracycline were also recommended for chlortetracycline and tetracycline.

Maximum Residue Limits
The Committee recommended that the MRLs for oxytetracycline of 100μg/kg in muscle, 300μg/kg in liver and 600μg/kg in kidney of cattle, pigs, sheep and poultry and of 100μg/l in milk (cattle and sheep) and 200μg/kg in eggs (poultry) be extended to chlortetracycline and tetracycline.

The Committee also recommended that the MRL of 10μg/kg for oxytetracycline in fat be withdrawn, and decided that MRLs for chlortetracycline and tetracycline in fat were not required.

With the above recommended MRLs, the theoretical maximum daily intake of chlortetracycline, oxytetracycline and tetracycline, used alone or in combination, would be 260μg, 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).

3.3.2 Oxytetracycline
Oxytetracycline was previously evaluated at the forty-fifth meeting of the Committee (Annex 1, reference 119), when a temporary MRL of 100μg/kg, expressed as parent drug, was assigned to the edible tissue of the giant tiger prawn (Penaeus monodon). The MRL was temporary pending the availability of a validated analytical method for the determination of oxytetracycline in prawns, which was required for review in 1996.

Residue data
An analytical method for the quantitative determination of oxytetracycline in the edible tissue of giant tiger prawns was submitted for consideration at the present meeting. The method is currently used for the determination of residues of chlortetracycline, oxytetracycline and tetracycline in muscle and kidney of cattle and pigs.

The results of the evaluation of the method as applied to oxytetracycline in prawn muscle included data from three analysts in two laboratories. It was found that the recovery of oxytetracycline from the muscle of giant tiger prawns at the temporary MRL established at the
forty-fifth meeting (100μg/kg) was 82–97%. The recovery at 50μg/kg was 60–91%. The coefficient of variability between the different analysts was considered acceptable (10–20%). These values are similar to those reported in other studies in different tissues.

Since the requested analytical method has been provided and is acceptable, the Committee recommended an MRL of 100μg/kg for oxytetracycline residues in muscle of giant tiger prawns.

3.3.3 Neomycin

Neomycin is an aminoglycoside antibiotic that was previously evaluated at the forty-third meeting of the Committee (Annex 1, reference III). At that time, the Committee established a temporary ADI of 0–30μg per kg of body weight, based on a NOEL of 6mg per kg of body weight per day for ototoxicity in a 90-day study in guinea-pigs and a safety factor of 200. The ADI was made temporary in view of deficiencies in the genotoxicity data. The in vitro genotoxicity data available at the forty-third meeting indicated that neomycin causes chromosomal aberrations, but only a limited number of studies were available and these had been poorly performed.

Toxicological data

At the present meeting, the Committee considered new data on genotoxicity, including the results of a reverse mutation assay in bacteria using Salmonella typhimurium and Escherichia coli, a forward mutation assay in Chinese hamster ovary cells, and an in vivo cytogenetic assay in mice. All gave negative results.

The Committee concluded that the results from these new tests indicated that neomycin is not genotoxic.

An ADI of 0–60μg per kg of body weight was established, based on the NOEL of 6mg per kg of body weight per day for ototoxicity in the guinea-pig and a safety factor of 100.

Residue data

No new residue-depletion studies were available. However, in view of the ADI established by the Committee at the present meeting, certain residue data were re-evaluated before MRLs were recommended. These data support the oral use of neomycin sulfate in certain food animals.

In a series of recently conducted residue-depletion studies in cattle, pigs, sheep and goats, neomycin sulfate was administered to the animals as a single daily dose equivalent to 15mg of neomycin base per kg of body weight for 14 days. No measurable residues of neomycin
were found in any of the samples of liver, muscle or fat taken at any time after the drug was withdrawn. The quantification limit was 0.5 mg/kg in these studies. Kidney was considered to be the target tissue in these species and parent neomycin was established as the marker residue. The concentration of neomycin found in the kidneys of cattle, pigs, sheep and goats at 1 day after withdrawal ranged from below the limit of detection up to about 4.2 mg/kg.

However, when a similar residue-depletion study was conducted in young (3-day-old) calves given a single daily dose equivalent to 15.4 mg of neomycin base per kg of body weight for 14 days, the depletion of the residues from kidney was found to be slow. Residue levels were 3.9–6.8 mg/kg in the kidneys of the four animals slaughtered at 28 days after withdrawal of the drug.

The Committee noted that measurable concentrations of neomycin residues had also been found in the livers of some animals in a 1967 study in which 15 female calves were given oral doses of neomycin sulfate, equivalent to 7.7 mg of neomycin base per kg of body weight per day, on 5 consecutive days. The concentrations were 2.75 mg/kg at 3 days after withdrawal, 1.01 mg/kg at 7 days, 0.62 mg/kg at 17 days and 1.7 mg/kg at 24 days. However, such findings have not been confirmed in the contemporary studies. The Committee concluded that the establishment of MRLs in edible tissues should be based on the results obtained in the contemporary studies, which were well designed and properly documented.

**Maximum Residue Limits**

At its forty-third meeting, the Committee recommended temporary MRLs because the ADI was temporary. The MRLs for cattle, sheep, goats, pigs, turkeys, ducks and chickens were 5000 μg/kg for kidney and 500 μg/kg for muscle, liver and fat, expressed as parent drug. The temporary MRLs recommended for chicken eggs and cows’ milk were 500 μg/kg and 500 μg/l respectively, expressed as parent drug.

At its present meeting, the Committee concluded that it was unnecessary to change these MRLs, with the exception of that for kidney, which — in order to establish practical withdrawal times for all target animal species — it was necessary to increase from 5000 μg/kg to 10,000 μg/kg.

The Committee recommended the following final MRLs for cattle, sheep, goats, pigs, turkeys, ducks and chickens: 10,000 μg/kg for kidney, and 500 μg/kg for muscle, liver and fat, expressed as parent drug. The final MRLs recommended for chicken eggs and cows’ milk were 500 μg/kg and 500 μg/l, respectively, expressed as the parent drug.
From the above MRL values, the calculated theoretical maximum daily intake of neomycin residues is 1525 µg, based on a daily food intake of 300 g of muscle, 100 g of liver, 50 g each of kidney and fat, 100 g of eggs and 1.51 of milk (Annex 1, reference 85). This is considerably less than the theoretical maximum ADI of 3600 µg of neomycin for a 60-kg person.

3.3.4 Spiramycin

Spiramycin is a macrolide antibiotic produced by certain strains of *Streptomyces ambofaciens* and used in oral or parenteral formulations for the treatment and prophylaxis of local and systemic bacterial and mycoplasmal infections in cattle and pigs.

It had been previously evaluated at the thirty-eighth and forty-third meetings of the Committee (Annex 1, references 97 and 113). At the time of those meetings, a validated chemical method was not available for the determination of spiramycin and neospiramycin residues in pig tissues, hence it was not possible to estimate the percentage of total antimicrobial activity accounted for by these two residues.

At its forty-third meeting, the Committee required the following information for evaluation in 1996:

1. A validated analytical method for determining the concentrations of spiramycin and neospiramycin in the edible tissues of pigs.
2. Residue data to estimate the percentage of the total antimicrobial activity accounted for by spiramycin and neospiramycin in the liver, kidney and fat of pigs.

*Analytical methods*

Data from studies using a microbiological method and an HPLC method for the determination of spiramycin and neospiramycin in pig tissues were provided.

*Microbiological method.* A microbiological method that was submitted for consideration at the forty-third meeting of the Committee (Annex 1, reference 113) was re-evaluated because additional information on the detection limit, the quantification limit and reproducibility was provided. The method, which is described in the *European pharmacopoeia* (7), was accepted at the forty-third meeting of the Committee for screening muscle tissues for evidence of spiramycin residues. In this method, a solvent extraction step is followed by an agar diffusion assay using *Micrococcus luteus* ATCC 9341 as the test organism. The quantification limits of the method, which were estimated using fortified tissues of a control pig, were 0.3 mg/kg for liver and kidney, 0.1 mg/kg for muscle, and 0.115 mg/kg for fat. Recoveries
were >80% for liver, kidney and muscle, but were lower (69%) for fat. When the method was used to examine tissues containing incurred residues at the recommended MRL, the coefficient of variation of the mean of 2–3 replicate analyses of the same sample was of the order of 20–25%, with a range of less than 5% to more than 50%. Analyses of liver tissues containing incurred residues slightly above the MRL showed that the results obtained with the microbiological assay were, on average, about 40% higher than those obtained with an HPLC method.

The microbiological method was specific for spiramycin in the presence of oxytetracycline and sulfadimidine, which may be formulated in feed together with spiramycin.

The Committee concluded that this microbiological method is suitable for use in screening pig tissues for residues of spiramycin and its active metabolites, providing that the results can be confirmed by a more specific method. However, the possibility of cross-reactivity with hydrophilic antibiotics cannot be excluded.

**HPLC method.** Data submitted from a number of studies were considered. An HPLC method using fortified samples of pig tissues demonstrated suitable sensitivity (detection limit 0.018 mg/kg and quantification limit 0.033 mg/kg) for analysing muscle tissue for spiramycin. Because of chromatographic interference, the method was found to be unsuitable for analysis of kidney or liver tissue. It was specific in the presence of tylosin.

A further HPLC study using spiked samples demonstrated that the quantification limits for spiramycin and neospiramycin in muscle samples were 0.025 mg/kg with recoveries of 93% and 100%, respectively. The quantification limit for liver was 0.2 mg/kg for spiramycin and 0.1 mg/kg for neospiramycin, with recoveries in the range 80–83%.

There was good correlation between the HPLC method and the microbiological assay as demonstrated in the analysis of liver tissues containing incurred residues in the range 0.1–5 mg/kg.

The Committee concluded that the HPLC methods were suitable for measuring spiramycin residues in muscle, liver and kidney at the level of the MRL. Tylosin did not interfere in the HPLC assay.

In an HPLC study on tissues from pigs fed spiramycin, the spiramycin and neospiramycin cysteine conjugates were found to account for 97.5% of the total residues, and for approximately 90% of the antimicrobial activity of the parent drug, thereby supporting the use of the microbiological method for routine screening.
**Maximum Residue Limits**

In the light of the recent decision of the Codex Committee on Residues of Veterinary Drugs in Foods (2) that a validated analytical method must be available for each MRL before the recommended MRLs may be presented to the Codex Alimentarius Commission for adoption as a food safety standard, the Expert Committee considered it appropriate to harmonize the MRLs for parent spiramycin residues in different tissues of different food-producing animals.

The recommended MRLs for spiramycin in cattle, pigs and chickens are shown in Table 3. For cattle and chickens, the MRLs are expressed as the sum of the concentrations of spiramycin and neospiramycin. For pigs, the MRLs are based on antimicrobial activity and are expressed as spiramycin equivalents.

If these values are used for the MRLs, the theoretical maximum daily intake of spiramycin residues would be 440 μg (see Table 4), based on

<table>
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<tr>
<th>Table 3</th>
<th>Recommended MRLs for spiramycin (μg/kg) in cattle, pigs and chickens</th>
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<tr>
<td><strong>Species</strong></td>
<td><strong>Recommended MRLs</strong></td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td>Cattle</td>
<td>200&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pigs</td>
<td>200&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Chickens</td>
<td>200&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

NA: not applicable.

<sup>a</sup> Expressed as μg/kg.

<sup>b</sup> Expressed as the sum of the concentrations of spiramycin and neospiramycin.

<sup>c</sup> Expressed as spiramycin equivalents.

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Theoretical maximum intake of spiramycin residues</th>
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<tbody>
<tr>
<td><strong>Tissue</strong></td>
<td><strong>Recommended MRL (μg/kg)&lt;sup&gt;a&lt;/sup&gt;</strong></td>
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<tr>
<td></td>
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</tr>
<tr>
<td>Muscle</td>
<td>200</td>
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<td>Liver</td>
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<td>Kidney (chickens)</td>
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<td>Fat</td>
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<tr>
<td>Milk</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
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</tbody>
</table>

Total | 440 |

<sup>a</sup> Expressed as the sum of the concentrations of spiramycin and neospiramycin.

<sup>b</sup> Based on a daily intake of 300 g of muscle, 100 g of liver, 50 g each of kidney and fat, and 1.5 l of milk.

<sup>c</sup> Spiramycin and neospiramycin accounted for 100% of the total residues in muscle and milk.

<sup>d</sup> Spiramycin and neospiramycin accounted for 50% of the total residues in liver, kidney and fat.

<sup>e</sup> Expressed in μg/kg.
a daily food intake of 300g of muscle, 100g of liver, 50g each of kidney and fat, and 1.51 of milk (Annex 1, reference 85).

Based on the ADI of 0–50µg per kg of body weight, the permitted daily intake of spiramycin would be 3000µg for a 60-kg person.

The Committee recommended that the current temporary MRLs for liver, kidney, fat and milk be made permanent.

3.3.5 Thiamphenicol

Thiamphenicol is an analogue of chloramphenicol. It is a broad-spectrum antibiotic administered orally to control infections in humans, pigs, poultry and young calves. Thiamphenicol is bacteriostatic against both Gram-positive and Gram-negative bacteria and some anaerobic organisms.

Thiamphenicol has not previously been evaluated by the Committee.

Toxicological and microbiological data

A range of studies on thiamphenicol were available for evaluation by the Committee, including data on pharmacokinetics, acute and short-term toxicity, reproductive and developmental toxicity, genotoxicity, and limited information on long-term toxicity. Studies on the microbiological effects of thiamphenicol and epidemiological data on humans were also considered by the Committee. The Committee noted that the protocols used in many of the studies would not meet contemporary standards, and therefore the substance was evaluated in accordance with the procedures developed for drugs with a long history of use (Annex 1, reference 104, section 2.3).

The pharmacokinetic data showed that the drug is rapidly absorbed when administered by the oral or parenteral routes (see p. 34).

Single oral doses of thiamphenicol were of low toxicity to mice and rats (LD₅₀ >3000mg per kg of body weight).

Short-term oral toxicity studies with thiamphenicol were performed in rats, dogs and pigs. In a 13-week study in rats dosed at 30, 45, 65 or 100mg per kg of body weight per day, increased mortality was observed among animals given 100mg per kg of body weight per day. In a 6-month study in rats, where the highest dose used was 120mg per kg of body weight per day, increased mortality was not reported. In both studies, a decrease in body-weight gain during treatment was seen at doses of 65mg per kg of body weight per day and above. Dose-related decreases in red blood cell parameters, differential and total white blood cell counts and clotting parameters were observed in the 13-week study, but not in the 6-month study. A reduction in the
number of germinal epithelial cells in the testes was seen at doses of 65 and 100 mg per kg of body weight per day in the 13-week study. The NOEL in this study was considered to be 30 mg per kg of body weight per day.

Dogs were given 40 or 80 mg of thiamphenicol per kg of body weight per day for 7 weeks. At both dose levels decreases in body weight were observed, as well as reversible decreases in erythrocyte volume fraction, haemoglobin and erythrocyte count. At 40 mg per kg of body weight per day, superficial erosion of the gall bladder mucosa was observed. The highest dose produced haemorrhagic ulcers in the gall bladder, diffuse mucous membrane enteritis and early involution of the thymus. Two out of four dogs in the lowest-dose group showed a reduction in the number of germinal epithelial cells in the testes and multinucleated cells in the seminiferous tubules.

In another study, thiamphenicol was given to dogs at doses of 30, 60 or 120 mg per kg of body weight per day for 4 weeks. Body weights of animals in the highest-dose group were slightly lower than those of controls. Increases in the absolute and relative liver weights and hepatocellular hypertrophy were observed at 60 and 120 mg per kg of body weight per day.

In a 6-month study, dogs were given thiamphenicol at doses of 15, 30 or 60 mg per kg of body weight per day. The body weights of males in the highest-dose group were up to 18% lower than those of controls during the study. The main haematological findings were decreases in red blood cell parameters at the highest dose level. Increases were noted in the mean serum cholesterol and phospholipid concentrations in males (30 and 60 mg per kg of body weight per day) and females (60 mg per kg of body weight per day) and the mean serum glucose concentration in males (60 mg per kg of body weight per day) and females (30 and 60 mg per kg of body weight per day). The relative liver weights were increased at 30 and 60 mg per kg of body weight per day. Histopathological lesions related to treatment were seen in the thymus (early involution), bone marrow (decreased cellularity), testes (focal and diffuse tubular atrophy) and oesophagus (ulceration) at the highest dose. The NOEL was 15 mg per kg of body weight per day.

In a 4-week study, pigs were treated with 25, 50 or 100 mg of thiamphenicol per kg of body weight per day. In the highest-dose group, a slight reduction in body-weight gain as well as reductions in mean packed cell volume, haemoglobin and erythrocyte counts were observed, and histological examination showed vacuolation in the epithelial cells of the renal tubules and mild diffuse vacuolation in
hepatocytes. A treatment-related reduction in urine pH was observed in all treated groups.

A summary report of a 2-year carcinogenicity study in rats, including a range-finding study, was available. Rats were given 125 or 250 mg/l of thiamphenicol in drinking-water (equal to 8 or 16 mg per kg of body weight per day for males and 10 or 19 mg per kg of body weight per day for females) for 104 weeks. The animals in the highest-dose group showed a decrease in body-weight gain, but there was no significant increase in the incidence of tumours in treated groups compared to controls.

In a long-term study in mice, which was designed primarily to investigate the effects of thiamphenicol on the immune response, thiamphenicol was administered orally at doses of 25, 50 or 250 mg per kg of body weight per day. No evidence of neoplastic or pre-neoplastic changes was observed.

In a study to determine the effect of thiamphenicol on fertility in rats, the drug was administered orally at doses of 120, 180 or 240 mg per kg of body weight per day to groups of 10 male rats. Five animals from each group were killed for histopathological examination after 4, 8 and 12 weeks, while the remainder were mated with untreated females at the given time intervals. Reductions in the number of germinal epithelial cells in the testes of all treated animals were observed. These changes were present up to 21 days after termination of treatment, but full recovery was observed by 50 days. The histological changes were correlated with the fertility index. Litters from matings between treated males and untreated females were normal in number and no physical abnormalities were reported.

Thiamphenicol was given orally to rats from day 15 of gestation to day 21 postpartum at doses of 30, 60 or 120 mg per kg of body weight per day. In the mid- and highest-dose groups, there were higher post-implantation losses and increased rates of perinatal mortality. The physical development of pups was inhibited during the lactation period in a dose-dependent manner. The sexual behaviour and fertility of F1 animals were normal, and animals in the F2 generation showed no abnormalities.

In four teratogenicity studies in rats, thiamphenicol was administered orally at doses of 40, 80 or 160 mg per kg of body weight per day from days 1 to 21 of gestation or at doses of 80 or 160 mg per kg of body weight per day during days 1–7, 7–14 or 14–21 of gestation. No teratogenic effects were observed. In all animals treated from days 1 to 21, a dose-related increase in resorption was noted and newborn pups had a higher mortality rate.
A teratogenicity study was performed in rabbits, which were given thiamphenicol orally at doses of 5, 30, 60 or 80 mg per kg of body weight per day on days 8–16 of gestation. Complete resorption of embryos occurred at 80 mg per kg of body weight per day. In other treated groups, moderate fetal toxicity and dose-related increases in abortion rate and resorption were reported. No fetal malformations were reported.

In another teratogenicity study, rabbits received thiamphenicol orally at doses of 1.25, 2.5 or 5 mg per kg of body weight per day on days 6–18 of gestation. Mild maternal toxicity was observed in animals in the mid- and highest-dose groups in the form of depressed body weights during treatment. No effects were observed on embryo or fetal development. The NOEL was 1.25 mg per kg of body weight per day.

Thiamphenicol gave negative results in five in vitro genotoxicity tests and in an in vivo micronucleus assay in mice.

The Committee considered data from human epidemiological studies and concluded that there is no evidence that thiamphenicol can induce aplastic anaemia, in contrast to the structurally related compound, chloramphenicol.

The Committee also considered data from several in vitro studies to determine the MIC values of thiamphenicol for a wide range of animal and human pathogens as well as genera representative of the human gut flora. The mean MIC₅₀ value was 1.68 µg/ml for 261 bacterial strains isolated from humans. The following genera were found to be the most sensitive: Bacteroides, Fusobacterium, Propionibacterium and Actinomyces. The Committee further noted that administration of 40 µg of thiamphenicol per kg in food to mice over 35 days did not alter the intestinal microflora in this species.

In calculating an ADI based on antimicrobial activity, the Committee used the formula developed at the thirty-eighth meeting (Annex 1, reference 97) and modified at the present meeting (see Annex 3):

\[
\text{Upper limit of ADI} = \frac{\text{MIC}_{50}(\mu\text{g/g}) \times \text{Mass of colonic contents (g)}}{\text{Fraction of oral dose} \times \text{Safety factor} \times \text{Body weight (kg)}}
\]

\[
= \frac{1.68 \times 220}{0.4 \times 1 \times 60}
\]

\[
= 15 \mu\text{g per kg of body weight}
\]
It took the following factors into consideration:

- Concentration: 1.68 µg per ml was the mean MIC₅₀ for microbiological effects on the human intestinal microflora.
- Availability: the fraction of the dose available to the gut microflora was derived from studies of thiamphenicol in humans, which showed that 60% of an oral dose is excreted unchanged in the urine within 24 hours. The fraction of the dose available in the intestinal tract is therefore 0.4.
- Safety factor: the Committee noted that a substantial amount of data covering a variety of bacterial strains representative of the human microflora and in vivo data from animal studies were available. It concluded that these data provide sufficient information on the microbiological effects of thiamphenicol. A safety factor of 1 was therefore adopted.

In reviewing the available toxicological and microbiological data and in considering the ADI based on antimicrobial activity, the Committee concluded that the toxicological data provided the most appropriate end-point for the evaluation of thiamphenicol. The Committee established a temporary ADI of 0–6 µg per kg of body weight for thiamphenicol, based on the NOEL of 1.25 mg per kg of body weight per day for maternal toxicity in the teratogenicity study in rabbits and a safety factor of 200. The ADI was designated as “temporary” because only a summary report of the carcinogenicity study in rats was available.

Metabolism and pharmacokinetic data
Thiamphenicol differs from chloramphenicol in that it is not readily metabolized in cattle, poultry, sheep or humans, but is predominantly excreted unchanged in the urine. In humans and animals given a single oral dose of 30 mg per kg of body weight, approximately 60% of the dose was excreted unchanged in the urine within 24 hours. In pigs, the drug is excreted both as parent drug and as thiamphenicol glucuronate.

Administration of a single oral dose of 100 mg/kg of thiamphenicol to rats and rabbits resulted in plasma levels of 32 and 6.8 mg/l, respectively, within 2 hours. Plasma levels were below the quantification limit (0.02 mg/l) at 14 hours after dosing. In rats given a single oral dose of 30 mg per kg of body weight of radiolabelled thiamphenicol, plasma concentrations were 6 mg/l 2 hours after dosing; by 48 hours after dosing, 62% of the dose had been eliminated in urine and 35% in faeces. After intravenous administration to rats, the half-life was estimated to be 46 minutes.
Administration of $[^14]C$thiamphenicol to broiler chicks as a single oral dose of 25 mg per kg of body weight or a single intravenous dose of 5 mg per kg of body weight resulted in peak plasma levels of 6.56 mg/l and 4.1 mg/l at 2 hours and 15 minutes after dosing, respectively. All plasma levels were at or below 0.02 mg/l at 24 hours after dosing. In another trial in which thiamphenicol was given in drinking-water for 3 days at dose rates of 15–67 mg per kg of body weight, plasma levels were dose-related, peaking at 3.75 mg/l and falling below 0.02 mg/l at 56 hours after dosing. Levels in liver, kidney and muscle were 0.07, 0.06 and 0.05 mg/kg, respectively, at 56 hours after dosing, and below 0.02 mg/kg at 104 hours after dosing.

In 16 calves dosed orally with unlabelled thiamphenicol at a dose of 25 mg per kg of body weight per day for 4 days, HPLC analysis showed that mean plasma levels of parent drug were 7.1 (range 5.0–9.2), 2.25 (1.85–2.65) and 0.54 (0.14–0.94) mg/l at 6, 24 and 34 hours after dosing, respectively.

Eight lactating cows were given unlabelled thiamphenicol intramuscularly at a dose rate of 15 mg per kg of body weight per day for 5 days. The mean drug levels in plasma peaked at 18 mg/l 30 minutes after the first dose and were 2.5 mg/l 6 hours after the first dose.

In another study, three groups of five pigs were given unlabelled thiamphenicol orally for 5 days at dose rates of 20, 30 or 40 mg per kg of body weight per day. The mean levels of parent drug in plasma peaked at 1.29 (range 0.5–2.08), 2.02 (1.58–2.46) and 2.81 (0.95–4.67) mg/l, respectively, within 2 hours of dosing. At all sampling times, thiamphenicol glucuronate levels were higher than those of unchanged drug. At 48 hours after the cessation of treatment, the mean thiamphenicol levels in plasma were 0.02 and 0.04 mg/l in the groups dosed at 20 and 40 mg/kg, respectively. No sampling was carried out more than 48 hours after dosing, so an end-point for thiamphenicol levels in plasma was not defined.

Twelve sheep were each given four intramuscular doses of thiamphenicol at 20 mg per kg of body weight at 8-hourly intervals. Plasma levels peaked at 20.4 mg/l within 30 minutes of dosing and were <0.01 mg/l (the detection limit) by 24 hours after dosing.

**Residue data**

Laying hens were fed a thiamphenicol-supplemented diet for 5 days which provided 56 mg of drug per hen per day. On the first day after dosing, the mean thiamphenicol level in egg homogenate was 0.27 mg/kg. At 7 days after dosing, the eggs from 7 of 15 hens had drug levels <0.02 mg/kg (quantification limit) and the remaining birds produced
eggs containing 0.02–0.04 mg/kg. All eggs had residues below the quantification limit at 9 days after dosing.

Residue-depletion studies in broilers given [14C]thiamphenicol by gavage at a dose rate of 25 mg per kg of body weight per day twice daily for 5.5 days showed that female birds had higher tissue drug levels than male birds. In females at 6 hours after dosing, bile contained 54 mg/l of parent drug equivalent, and levels in liver, kidney and skin were 8.5, 5.5 and 1.4 mg/kg, respectively. At 120 hours after dosing, residue levels in liver, kidney and skin in females were 1.1, 0.9 and 0.4 mg/kg, respectively.

In the study in 16 calves dosed orally at 25 mg per kg of body weight per day for 4 days, thiamphenicol concentrations in muscle were below the quantification limit (0.02 mg/kg) at 6 days after dosing, and levels in liver and kidney were below the quantification limit by the eighth day after dosing.

In lactating cattle dosed intramuscularly at 30 mg per kg of body weight per day for 5 days, thiamphenicol levels in milk were 2.4 mg/l on day 1 of dosing. One day after dosing, thiamphenicol levels in milk were 0.76 mg/l. Levels in milk were <0.02 mg/l (the quantification limit) in 6 of the 8 cows on the second day after dosing and in all the cattle on the fourth day after dosing.

Six groups of two pigs were dosed orally with unlabelled thiamphenicol for 5 days at a dose rate of 40 mg per kg of body weight per day. There was considerable variation in the levels of parent drug in tissue. The concentration in fat was <0.02 mg/kg (quantification limit) on the tenth day after dosing and that in muscle was below the quantification limit on the eleventh day. The concentration in liver fell below the quantification limit on the fifteenth day, at which time levels in kidney were 0.3 mg/kg. No further samples were collected, so the end-point for thiamphenicol levels in kidney could not be determined. The Committee concluded, however, that this study was not adequate for the assessment of residues in pigs.

Analytical methods
Adequate analytical methods are available, generally involving HPLC with UV detection or gas–liquid chromatography (GLC) with electron-capture detection. Recoveries of over 90% and quantification and detection limits of 0.02 mg/kg and 0.01 mg/kg, respectively, have been reported.

Maximum Residue Limits
In reaching its decision on MRLs, the Committee took the following factors into account:
• The temporary ADI of 0–6μg/kg of body weight, which is based on a toxicological end-point. This corresponds to 360μg for a 60-kg person.
• The absence of data to determine the percentage of the total residues accounted for by the marker residue in the edible tissues of target species.
• The quantification and detection limits of the available analytical methods (0.02mg/kg and 0.01mg/kg, respectively).
• The lack of residue-depletion studies in target animals covering periods longer than the recommended withdrawal times at the maximum recommended dosage.

The Committee recommended temporary MRLs for thiamphenicol of 40μg/kg for muscle, liver, kidney and fat, expressed as parent drug, in poultry and cattle. These temporary MRLs were derived by doubling the quantification limit of the available analytical methods.

MRLs were not recommended for eggs because of the unacceptably high thiamphenicol residues. No MRLs were proposed for cows’ milk or pigs, as no data were submitted on total residues in milk and the residue data on pigs were inadequate.

From these values for the MRLs, the maximum theoretical intake would be 20μg per day, based on a daily intake of 300g of muscle, 100g of liver, and 50g each of kidney and fat (Annex 1, reference 85). This is well below the quantity permitted by the ADI of 360μg for a 60-kg person.

The following information is required for evaluation in 1999:
1. Detailed reports on the carcinogenicity study in rats for which the summary report was available at the present meeting and on the range-finding study used to establish dose levels in that study.
2. Residue-depletion studies with radiolabelled and unlabelled thiamphenicol for the identification of the marker residue and target tissues in poultry, pigs and young (non-ruminant) calves.

3.3.6 Tilmicosin

Tilmicosin is a macrolide antibiotic developed from tylosin for veterinary use, available as an injectable formulation for cattle and sheep and as a premix for swine feeds. The commercial product is a mixture of the cis (82–88%) and trans (12–18%) isomers. Tilmicosin is recommended for the treatment and prevention of pneumonia in cattle, sheep and pigs.

Tilmicosin has not been previously evaluated by the Committee.
Toxicological and microbiological data

The Committee considered the results of studies on pharmacokinetics (see p. 41), metabolism, acute and short-term toxicity, reproductive toxicity, teratogenicity, genotoxicity, antimicrobial activity and pharmacology. The Committee also considered observations in humans accidentally exposed to tilmicosin.

In metabolism studies (see p. 41), the metabolites detected in the target species were also found in rats, which suggests that the rat is a suitable model for determining the potential toxicological hazards posed by tilmicosin.

The LD₅₀ in fasted rats was 800–850 mg per kg of body weight, but toxicity was substantially lower in non-fasted animals, among which there were no deaths following administration of a single dose of 2000 mg of tilmicosin per kg of body weight. Studies in a range of pharmacological models identified depression of contractility of cardiac muscle and reduced heart function as the main adverse effects.

Rats were given oral doses of 50, 250 or 1000 mg of tilmicosin per kg of body weight per day for 3 months. At 1000 mg per kg of body weight per day, animals exhibited chromorrhinorrhoea, chromodacryorrhoea, alopecia, poor grooming and reduced food consumption, and they appeared emaciated. Body-weight gain was reduced and mortality was increased in females at 250 mg per kg of body weight per day and in both sexes at 1000 mg per kg of body weight per day. Toxic effects on the kidney and liver were indicated by increased organ weights, increased levels of serum alanine aminotransferase and blood urea nitrogen, and the presence of blood in the urine. However, pathological examination of these organs showed only slight nephrosis in two males out of 20 in each of the groups given 250 or 1000 mg per kg of body weight per day. At the highest dose, hypertrophy of the zona fasciculata of the adrenal cortex was noted in most rats, and myocardial degeneration, necrosis of individual skeletal muscle fibres and lymphoid depletion in the spleen and thymus were seen in some rats. The NOEL was 50 mg per kg of body weight per day.

Dogs were given oral doses of 6, 20 or 70 mg of tilmicosin per kg of body weight per day for 3 months. Of the 8 males and females given 70 mg per kg of body weight per day, 4 died during the first month. Heart rate was increased at 20 and 70 mg per kg of body weight per day and at the higher dose there was depression of the S-T segment of the electrocardiogram. Ophthalmoscopic examination of the eyes showed bilateral areas of subretinal fluid concentrated along arterioles in the tapetal region of 2 of the 4 surviving dogs at 70 mg per kg
of body weight per day. The NOEL was 6mg per kg of body weight per day.

Dogs were given oral doses of 4, 12 or 36mg of tilmicosin per kg of body weight per day for 12 months. Body-weight gains were depressed at 12 and 36mg per kg of body weight per day. Heart rates were markedly increased at 36mg per kg of body weight per day, and there was occasional depression of the S–T segment of the electrocardiogram. Enlargement of the heart was also noted at the highest dose, but there were no associated pathological changes. The NOEL was 4mg per kg of body weight per day.

In a 2-generation reproductive toxicity study, rats were given oral doses of 10, 45 or 200mg of tilmicosin per kg of body weight per day. Body-weight gain and food consumption were depressed in females given 45 and 200mg per kg of body weight per day, but only during the premating period. Fertility and reproductive parameters were unaffected at any dose level. At 200mg per kg of body weight per day, mortality in pups was slightly increased in both F1 litters up to 4 days postpartum, but not in either F2 litter. The NOEL was 10mg per kg of body weight per day.

Developmental toxicity studies in rats and rabbits were reported. Rats were dosed with 10, 70 or 500mg of tilmicosin per kg of body weight per day by gavage. The growth and development of rat fetuses were not affected by treatment, but increased salivation and reduced body-weight gain of dams were observed at 70 and 500mg per kg of body weight per day. The NOEL for maternal toxicity in rats was 10mg per kg of body weight per day. In the study in rabbits, there were dose-related signs of toxicity in the does at all doses (8, 19 and 48mg per kg of body weight per day). In the groups given 19 or 48mg per kg of body weight per day by gavage, does showing reduced body weights produced offspring that had open eyelids and low incidences of cleft palate or club foot. These fetuses had low body weights and were clearly retarded in development. The effects were attributed to treatment-related malnutrition in the does, which is commonly observed in rabbits dosed with antibiotics, confirming that this species is an inappropriate model for studying the teratogenic potential of antibiotics.

Tilmicosin has been tested in reverse mutation assays in bacteria, forward mutation assays in cultured mammalian cells, unscheduled DNA synthesis in primary cultures of rat hepatocytes, and in vivo cytogenetic assays. All results were negative, and the Committee concluded that tilmicosin has no genotoxic potential.
The carcinogenic potential of tilmicosin has not been tested. However, a number of factors are relevant in assessing the carcinogenicity of this drug. Toxicity studies with tilmicosin have not resulted in lesions or proliferative changes suggestive of neoplasia, and results were uniformly negative in a wide range of genotoxicity assays. Tilmicosin is a macrolide antibiotic, and this class of chemicals is not known to induce neoplasia despite widespread use in humans over many years. The closest structural analogue, tylosin, was reviewed at the thirty-eighth meeting of the Committee (Annex 1, reference 97).

In a 2-year feeding study in rats, tylosin administration was associated with an increased incidence of pituitary adenomas in male animals when compared to concurrent controls. New evidence, not considered at the thirty-eighth meeting, indicates that pituitary neoplasms occur spontaneously in male rats at variable rates, and that incidence rates increase with body weight. In male rats treated with tylosin, the 12-month body weights were somewhat higher than those in the control groups, and the incidences of pituitary adenomas were comparable to the upper limit of the range in historical controls. The lower incidence of pituitary neoplasms in the control animals may have been due to the earlier mortality in this group caused by respiratory disease. In the light of this information the Committee considered that the concerns of the thirty-eighth meeting about the potential tumorigenicity of tylosin had been satisfactorily addressed. For the above reasons, the Committee considered that carcinogenicity studies would not be required for tilmicosin.

In assessing the microbiological activity of tilmicosin, the Committee considered that the results of a study using germ-free rats colonized with human intestinal microflora were the most relevant. At the highest dose administered (0.4 mg per kg of body weight per day for 5 days) there were no significant alterations in the numbers of total anaerobes or enterobacteria in rat faeces.

There have been 36 cases reported of local reactions indicative of an irritant action following accidental dermal and ocular exposure to tilmicosin. People accidentally injected with tilmicosin have reported anxiety, sweating, headache and lightheadedness, but changes in the electrocardiogram pattern were observed in only one person. Contact with the buccal mucosa or ingestion has resulted in a range of symptoms, including nausea, vomiting, thirst, headache and numbness or a burning sensation in the mouth.

The Committee concluded that the most sensitive toxicological effects caused by tilmicosin were those in the 12-month study in dogs, in which the NOEL was 4 mg per kg of body weight per day. Administra-
tication of tilmicosin at a dose of 0.4 mg per kg of body weight per day to rats colonized with human intestinal microflora did not produce a significant antimicrobial effect. The Committee established an ADI of 0–40 μg per kg of body weight, based on the toxicological NOEL of 4 mg per kg of body weight per day and a safety factor of 100. It noted that an identical ADI would have been established based on the data from the microbiological study in rats colonized with human intestinal microflora and a safety factor of 10 to account for variability between humans.

Pharmacokinetic data
Tilmicosin is absorbed from the gastrointestinal tract as shown by the recovery of radiolabelled compound in the urine and/or bile of pigs dosed orally (110–400 mg per animal), and the presence of dose-related serum concentrations of tilmicosin in dogs dosed orally for 3 months. However, the results of the available studies did not allow any conclusion to be reached concerning the extent of absorption.

Absorption was similar after subcutaneous and intramuscular administration in calves. This was also observed in mature cattle, where absorption was slower than in calves. Following subcutaneous treatment, more than 75% of the dose was eliminated within 7 days in cattle and sheep, and there were similar findings in pigs treated orally. Residues at 7 days were found predominantly in the faeces in pigs, sheep and cattle (64.5%, 71.9% and 63.5% of the dose, respectively), with lesser amounts in the urine (5.6%, 13.3% and 22.7%, respectively). In sheep and cattle which received the recommended dose (10 mg per kg of body weight administered subcutaneously), residues in serum peaked at 0.44 mg/l at 8 hours after treatment and 0.97 mg/l at 6 hours, respectively; in neonatal calves, the levels peaked at 1.55 mg/l at 1 hour after treatment.

Metabolism data
Metabolism studies were conducted with [14C]tilmicosin in cattle, pigs, sheep and rats. The parent compound and a major metabolite identified as N-desmethyl tilmicosin were found in the faeces or urine of all four species.

Residue data
In three studies, [14C]tilmicosin was used to determine the distribution and depletion of the residues in cattle. In liver, following the administration of [14C]tilmicosin at 10 mg per kg of body weight by subcutaneous injection, the proportion of the total residues accounted for by the parent drug fell from 37% at day 3 to 7% at day 28. In muscle, the parent drug accounted for 45% of the total residues at day 3. Total
residues in muscle and fat were <0.10 mg/kg by day 14, approximately half of which were accounted for by the parent compound. Residues of the parent compound at the injection site were in the range 2–3 mg/kg (approximately half the total residues) at 28 days after treatment.

Treatment of lactating dairy cattle with tilmicosin is not recommended. However, in a separate study, milk collected from cattle which received a single subcutaneous injection of 10 mg of tilmicosin per kg of body weight contained residues in excess of 0.05 mg/kg for up to 3 weeks following administration.

In sheep which received a single subcutaneous injection of 20 mg of $[^{14}C]$ilmicosin per kg of body weight (twice the recommended dose), residues of parent drug in kidneys declined from 12.41 mg/kg at day 3 to 0.47 mg/kg at day 21. Residues of the parent drug were consistently higher in kidney than in liver at all sampling times except at 28 days in this study. Total residues were higher in liver than in kidney by day 7 and at subsequent sampling times. On the basis of this study, the parent compound was estimated to account for 8% and 33% of the total residues in liver and kidney, respectively, at 20 days after withdrawal. At earlier sampling times, when residues were detectable in muscle and fat, the parent compound was estimated to account for half of the total residues in these tissues, similar to the proportions found in cattle. Total and parent drug residues were 1.32 and 0.12 mg/kg, respectively, at the injection site at 28 days.

Similar results were obtained in a study in sheep, which received unlabelled tilmicosin subcutaneously at the recommended dose of 10 mg per kg of body weight. Residues of parent drug were 0.11 and 0.16 mg/kg in liver and kidney, respectively, at 14 days following administration and 0.07 mg/kg in both tissues at day 21. Traces of tilmicosin were found in some fat samples, but no residues were detected in muscle at day 14 or in later samples. At day 28, residues of parent drug were 0.06–0.13 mg/kg at the injection sites.

Three studies were conducted in pigs given $[^{14}C]$ilmicosin at 400 or 600 mg/kg in the feed. Animals dosed at 600 mg/kg (equal to 23 mg per kg of body weight per day) had total residues at the time of withdrawal of 12.3, 10.6 and 1.1 mg/kg in kidney, liver and muscle, respectively. Analysis by HPLC demonstrated that the parent compound accounted for >90% of the total residues. Residues of the parent compound declined to 0.14 and 0.07 mg/kg in liver and kidney, respectively, at 28 days and were <0.05 mg/kg in muscle and fat at day 7. The parent drug accounted for approximately half of the total residues in all tissues at 7, 14 and 28 days after dosing. Animals which received the recommended dose of 400 mg/kg (equal to 18 mg per kg of body
weight per day) had residue levels which were approximately half those found with the 600 mg/kg dose. Similar results were obtained in a separate study in pigs in which unlabelled tilmicosin was administered in the feed at the recommended dose of 400 mg/kg.

In lactating ewes which received a single subcutaneous injection of 10 mg of tilmicosin per kg of body weight, residues in milk depleted from 10.3 mg/l at 8 hours to 0.06 mg/l at day 10. Samples collected at days 14 and 21 were below the quantification limit of 0.05 mg/l of the HPLC assay.

Analytical methods
Several HPLC methods with ultraviolet detection have been used for the analysis of tilmicosin residues in the various edible tissues (muscle, liver, kidney and fat) and in sheep milk. For these assays, quantification limits of 0.005–0.02 mg/kg have been reported, depending on the tissue, species and the purification procedure used. Recoveries of 70% or above have generally been achieved.

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

- The ADI is 0–40 µg per kg of body weight, which is equivalent to a maximum daily intake of 2400 µg for a 60-kg person.
- Residues other than the parent drug were not fully characterized in the residue-depletion studies and must therefore be considered.
- Liver and muscle are the appropriate target tissues.
- The appropriate marker residue in all tissues is the parent drug.
- Residue-depletion studies using the radiolabelled drug are available for sheep tissues but no such study has been performed in lactating sheep.
- Suitable analytical methods are available for the marker residue.
- The available data indicate that the parent drug accounts for 5% of the total residues in cattle and sheep liver, 10% of the total residues in sheep kidney, 25% of the total residues in cattle kidney, and 50% of the total residues in pig liver and kidney, sheep milk, and muscle and fat (cattle, sheep and pigs). The proportion of total residues accounted for by the parent drug in sheep milk was estimated from ratios in muscle and fat.

The Committee recommended MRLs for tilmicosin of 100 µg/kg for muscle, 1000 µg/kg for liver, 300 µg/kg for kidney and 100 µg/kg for fat in both cattle and sheep, as well as 50 µg/l for ewes' milk, expressed as parent drug. The recommended MRL for ewes' milk is temporary, pending the receipt of the results of a study using radiolabelled drug.
in lactating sheep aimed at determining the relationship between total residues and the parent drug in milk. The Committee also recommended MRLs of 100μg/kg for muscle, 1500μg/kg for liver, 1000μg/kg for kidney and 10μg/kg for fat, expressed as parent drug, in pigs.

From these values for the MRLs, the maximum theoretical intake would be 2370μg per day (Table 5).

The Committee drew attention to the potential for residues in excess of the MRLs for muscle to exist at injection sites.

The results of a study in lactating sheep with radiolabelled tilmicosin aimed at determining the relationship between total residues and the parent drug in milk are required for evaluation in 1999.

3.4 Insecticides
3.4.1 Cypermethrin and α-cypermethrin

Cypermethrin is a synthetic pyrethroid insecticide applied topically to cattle, sheep and poultry. It is a mixture of four cis and four trans isomers, of which the cis isomers are the more biologically active and more persistent. α-Cypermethrin consists essentially of the two most active cis isomers of cypermethrin and is applied as a pour-on preparation for cattle and sheep, as a dip for sheep, and as a spray for poultry. A typical sample of cypermethrin contains 25% α-cypermethrin.

Cypermethrin and α-cypermethrin have not been previously evaluated by the Committee. Cypermethrin was evaluated by the Joint

<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 tilmicosin equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>100</td>
<td>200*</td>
<td>60</td>
</tr>
<tr>
<td>Liver</td>
<td>1000</td>
<td>20000*</td>
<td>2000</td>
</tr>
<tr>
<td>Kidney</td>
<td>300</td>
<td>3000*</td>
<td>150</td>
</tr>
<tr>
<td>Fat</td>
<td>100</td>
<td>200*</td>
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</tr>
<tr>
<td>Milk</td>
<td>50*</td>
<td>100*</td>
<td>150</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>2370</td>
</tr>
</tbody>
</table>

*Expressed as parent drug.
*Based on a daily intake of 0.5kg of meat made up of 300g of muscle, 100g of liver, 50g each of kidney and fat, and 1.5l of milk.
*The marker residue accounted for 50% of the total residues in muscle, fat and milk.
*The marker residue accounted for 5% of the total residues in liver.
*The marker residue accounted for 10% of the total residues in kidney.
*Expressed in μg/kg.
FAO/WHO Meeting on Pesticide Residues in 1979 and 1981 (8, 9), and an ADI of 0–0.05 mg per kg of body weight was established in 1981.

Toxicological data
The Committee considered data from a range of toxicological studies on cypermethrin and \( \alpha \)-cypermethrin, including the results of studies on their pharmacokinetics and metabolism, acute and short-term toxicity, reproductive toxicity, genotoxicity, long-term toxicity/carcinogenicity and neurotoxicity. Reports of effects in humans were also considered.

Cypermethrin and \( \alpha \)-cypermethrin are \( \alpha \)-cyano or type II pyrethroids that cause neurotoxicity in mammals and insects. They affect the sodium channels of nerve membranes, causing a long-lasting prolongation of the normally transient increase in sodium permeability of the membrane during excitation. At high dose levels, these pyrethroids induce salivation and tremors that progress to characteristic tonic–clonic convulsions (choreoathetosis and salivation syndrome).

After oral administration, cypermethrin is readily absorbed, distributed and excreted in rats, chickens, sheep and cattle (see p. 48). Studies in cattle indicated that absorption, distribution and excretion were comparable for cypermethrin and \( \alpha \)-cypermethrin (see pp. 48–49).

The acute oral toxicity of cypermethrin and \( \alpha \)-cypermethrin is moderate to high. WHO has classified these substances as "moderately hazardous". In rats and mice, the oral LD\(_{50}\) ranges from 82 to 4000 mg per kg of body weight for cypermethrin and from 35 to >5000 mg per kg of body weight for \( \alpha \)-cypermethrin, depending on the vehicle used. At lethal or near lethal doses, the signs are typical of type II pyrethroids, and include salivation, ataxia, gait abnormalities and convulsions.

Several short-term oral toxicity studies with cypermethrin were available, including 5-week and 90-day studies in rats and 5-week and 13-week studies in dogs. The dose levels ranged from 25 to 1600 mg/kg in the diet (equivalent to 1.2–80 mg per kg of body weight per day) in rats and from 5 to 1500 mg/kg in the diet (equivalent to 0.12–38 mg per kg of body weight per day) in dogs. In these studies, the clinical signs included ataxia, abnormal gait, nervousness and, particularly in dogs, lack of appetite, diarrhoea, vomiting and hyperaesthesia. In both rats and dogs, cypermethrin caused decreases in body-weight gain, food intake and a number of haematological parameters, increases in some organ weights and plasma urea levels, and, at lethal or near lethal doses, effects on the nervous system. For cypermethrin, the lowest
NOEL identified was in a 90-day study in rats given 25, 100, 400 or 1600 mg/kg in the diet (equivalent to 1.25–80 mg per kg of body weight per day). Male rats dosed at 1600 mg/kg in the diet showed decreases in haemoglobin, mean corpuscular volume and eosinophils, and increases in prothrombin time, plasma urea levels and relative liver and kidney weights. A decrease in eosinophils and an increase in relative liver weight were also observed in males dosed at 400 mg/kg in the diet. In female rats, reduced food intake and increased relative liver weights were noted at 1600 mg/kg in the diet. The NOEL in this study was 100 mg/kg in the diet, equivalent to 5 mg per kg of body weight per day.

The Committee evaluated a number of short-term oral toxicity studies on α-cypermethrin, including 29-day and 13-week studies in mice, 5-week, 6-week and 90-day studies in rats, and 13-week and 52-week studies in dogs. The dose levels ranged from 50 to 1600 mg/kg in the diet (equal to 7–230 mg per kg of body weight per day) in mice, 20 to 1200 mg/kg in the diet (equal to 1.2–60 mg per kg of body weight per day) in rats and from 30 to 270 mg/kg in the diet (equivalent to 0.75–6.8 mg per kg of body weight per day) in dogs. In these studies, α-cypermethrin caused the same effects as those described for cypermethrin in the short-term studies. The signs of toxicity included ataxia, abnormal gait, increased sensitivity to noise, hyperactivity, hunched posture and, as demonstrated histologically, axonal degeneration of the sciatic nerves. For α-cypermethrin, the lowest NOEL was identified in a 52-week study in dogs given a diet containing 60, 120 or 240 mg/kg (equivalent to 1.5–6 mg per kg of body weight per day). Dogs given 120 and 240 mg/kg in the diet showed reddening, ulceration and necrosis of the skin. The NOEL in this study was 1.5 mg per kg of body weight per day.

In a 3-generation reproductive toxicity study with cypermethrin, rats were dosed at levels of 10, 100 or 500 mg/kg in the diet (equivalent to 0.5–25 mg per kg of body weight per day). Animals in the highest-dose group showed reductions in body-weight gain and food consumption and there were concomitant reductions in litter size and weight in the first litter of the first generation only. No other effects on fertility or reproductive parameters were observed. The NOEL was 100 mg/kg in the diet, equivalent to 5 mg per kg of body weight per day.

Cypermethrin did not cause embryotoxicity or teratogenicity in rats at doses of up to 70 mg per kg of body weight per day or in rabbits at doses of up to 120 mg per kg of body weight per day. α-Cypermethrin did not cause embryotoxicity or teratogenicity in rats at dose levels of
up to 9 mg per kg of body weight per day or in rabbits at dose levels of up to 30 mg per kg of body weight per day. The NOELs for maternal toxicity in rats were 18 and 9 mg per kg of body weight per day for cypermethrin and α-cypermethrin, respectively, while those for maternal toxicity in rabbits were 30 and 3 mg per kg of body weight per day for cypermethrin and α-cypermethrin, respectively.

Cypermethrin and α-cypermethrin have been tested in a wide variety of in vitro and in vivo genotoxicity studies. All of the results were negative.

Two long-term toxicity/carcinogenicity studies on cypermethrin in mice and rats were available. Mice received a diet containing 100, 400 or 1600 mg/kg (equal to 14–230 mg per kg of body weight per day) for 101 weeks. In the highest-dose group, decreases in body-weight gain, changes in haematological parameters and increased liver weight were observed. The NOEL was 400 mg/kg in the diet, equal to 57 mg per kg of body weight per day. In a study in which rats received diets containing 1, 10, 100 or 1000 mg/kg (equivalent to 0.05–50 mg per kg of body weight per day) for 2 years, the only effects observed were reductions in body weight and food consumption at the highest dose. The NOEL was 100 mg/kg in the diet, equivalent to 5 mg per kg of body weight per day.

In a 2-year study, dogs received diets containing cypermethrin at 3, 30, 300 or 1000 mg/kg (equivalent to 0.075–25 mg per kg of body weight per day). The highest dose level was reduced to 600 mg/kg in the diet due to severe intoxication. The NOEL in this study was 300 mg/kg in the diet, equivalent to 7.5 mg per kg of body weight per day. The Committee concluded that cypermethrin was not carcinogenic in these studies.

No long-term toxicity/carcinogenicity or reproductive toxicity studies on α-cypermethrin were available. The Committee noted the absence of reproductive toxicity and carcinogenicity associated with the administration of cypermethrin which contains 25% of α-cypermethrin. The Committee also noted the absence of genotoxicity with both cypermethrin and α-cypermethrin, the absence of carcinogenicity associated with compounds of similar structure, and the similar metabolism and disposition of the two compounds. Taking all of these considerations into account, the Committee concluded that it was unnecessary to request the results of long-term toxicity/carcinogenicity or reproductive toxicity studies on α-cypermethrin.

Several studies on the neurotoxicity of cypermethrin and α-cypermethrin in rats were available. In these studies, high oral doses
of cypermethrin and α-cypermethrin caused clinical signs that included coarse tremor and spasmodic movements of the body and tail. Axonal damage in the sciatic and posterior tibial nerves and the trigeminal nerve and ganglion was indicated by significant increases in β-glucuronidase and β-galactosidase in nerve tissue homogenates, in addition to abnormal neuromuscular function tests. In the inclined plane test, cypermethrin in dimethylsulfoxide caused transient functional impairment. The lowest NOEL for neurotoxicity was 38 mg per kg of body weight per day for cypermethrin (in dimethylsulfoxide) and 4 mg per kg of body weight per day for α-cypermethrin (in corn oil), indicating that the toxicity may be influenced by the vehicle used.

Humans occupationally exposed to cypermethrin developed skin sensations as a first reaction, followed by systemic effects such as dizziness, headache, nausea, paraesthesia and increased sweating. In more serious cases, muscular fasciculations in large muscles or in the extremities developed. In experiments with operators spraying cypermethrin, no clinical signs of nervous system abnormalities were observed. However, exposure levels were not measured.

The Committee established an ADI of 0–50 µg per kg of body weight for cypermethrin, based on the NOEL of 5 mg per kg of body weight per day in the 90-day, 2-year and reproductive toxicity studies in rats and a safety factor of 100. The Committee established an ADI of 0–20 µg per kg of body weight for α-cypermethrin, based on the NOEL of 1.5 mg per kg of body weight per day in the 52-week study in dogs and a safety factor of 100. The ADI for α-cypermethrin was rounded to one significant figure, in keeping with standard practice (Annex 1, reference 91, section 2.7).

Metabolism and pharmacokinetic data

Cypermethrin. Following oral administration of [14C]cypermethrin to cattle, poultry or rats, the radioactivity was excreted rapidly in both the urine and faeces. Less than 1% of the dose was found in milk or eggs. When [14C]cypermethrin was applied topically to sheep, 2.5% of the dose was eliminated in the urine and faeces within 6 days; following oral dosing, about 60% of the dose was eliminated within 2 days.

After oral administration of [14C]cypermethrin, there was evidence of hydrolysis of the ester bond, and residues of each half of the molecule were found in different proportions in rats, cattle, poultry and humans. There was extensive metabolism in bovine liver and kidney, the major metabolites consisting either of the conjugates or free forms of
3-phenoxybenzoic acid or 4-hydroxy-3-phenoxybenzoic acid. Of the residues in liver and kidney, 97% and 92%, respectively, were extractable at room temperature. Bound residues in kidney were less than 10µg/kg and were not further investigated. The residues in milk and fat were 90% and 98% extractable, respectively, and shown to be cypermethrin. In fat and milk, the cis and trans isomers were present in equal amounts, which suggests that no interconversion of the isomers occurs. No information on metabolism following absorption through the skin of cattle or poultry was provided and residue profile data were not available for sheep.

α-Cypermethrin. In rats, 50–60% of a radiolabelled dose was excreted in the urine and 30–40% in the faeces over an 8-day collection period. When [14C]α-cypermethrin was administered orally to one lactating cow, most of the radioactivity was excreted in the faeces and urine (34% and 23%, respectively), whereas secretion in milk accounted for <1%. The liver and kidney contained at least eight and nine metabolites, respectively, with a broad range of polarities. One component, which accounted for 16% and 20% of the total residues in liver and kidney, respectively, had chromatographic properties similar to those of α-cypermethrin. Muscle, fat and milk contained mainly a single component which accounted for 85%, 91% and 97% of the extracted residues respectively and had chromatographic properties similar to those of α-cypermethrin. The metabolic profiles were not determined in sheep or poultry.

Residue data
Cypermethrin. Several residue-depletion studies were available, in which radiolabelled cypermethrin was given to cattle and poultry by the oral route and to sheep as a pour-on formulation. However, residues were measured only up to 1 day after the last dose in cattle and poultry and at 1 and 6 days after dosing in sheep. Moreover, the study in sheep was limited to two animals. It was therefore not possible to determine the depletion of the total residues in these species.

Studies in farm animals dosed with the radiolabelled compound showed that the residue levels were higher in fat, liver and kidney (up to 410µg/kg) than in muscle (up to 60µg/kg). When sheep were treated with the pour-on preparation, residue levels in the subcutaneous fat at the site of application were more than 10-fold higher than following an oral dose.

On the basis of the limited data available, the Committee concluded that cypermethrin is the only residue possible for selection as a
marker compound. It is suitable for measuring residues in fat, milk (in the butterfat) and eggs. However, the relationship between the concentration of cypermethrin and the total residues in muscle, liver and kidney was imprecise and not studied in the post-dosing period.

In a large number of trials the residues of cypermethrin were measured in cattle, sheep and poultry following the recommended field uses. In all these studies residues were lower in muscle, liver and kidney than in fat tissues or milk fat.

The residues in cattle following the use of ear tags impregnated with cypermethrin were mostly below the determination limit. The residues in cattle and hens treated with a spray formulation were at low levels (<10–30µg/kg) in muscle, liver and kidney in both species and in hen eggs. Residue levels were higher in fat (90µg/kg at 7 days after dosing) and in butterfat (110µg/kg at 4 days after dosing), but had declined to a mean value of 30µg/kg by day 10.

The residues of cypermethrin following the dipping of cattle with the commercial formulations were either below or very close to the quantification limit (10µg/kg) for muscle, liver and kidney. Residues were present in renal, omental and subcutaneous fat. The concentration in fat was still above the quantification limit by 14 days after dosing, the last sampling time studied. However, the highest residue in fat was 180µg/kg, most of the values being less than 100µg/kg.

Calves treated with 0.5 g of cypermethrin as a pour-on preparation had low or undetectable levels of residues in muscle and liver throughout the 14-day post-treatment study period. Residues (up to 130µg/kg) were present in the kidneys throughout the study period, and much higher levels were found in both peritoneal and subcutaneous fat. Concentrations of up to 1400µg/kg in peritoneal fat were the highest recorded for any treatment. The residues, although still present at 14 days, had declined to 330µg/kg. Since the dose used in this study (0.5 g per 125-kg calf) was the same as that recommended for larger, mature animals, residues in larger animals may be lower. In lactating cattle given a dose of either 0.5 g or 1 g of cypermethrin as a pour-on preparation, the residues in whole milk followed a predictable pattern, with the highest values (100µg/l on day 3) for the higher dose and all values declining to control values (2µg/l) within 21 days.

In sheep, the residues were measured following the application of either dip or pour-on preparations. In all the studies residues of cypermethrin in muscle, liver and kidney were close to, or in most cases below, the quantification limit. Residues were found in both the
perirenal fat (<10–150 \mu g/kg) and omental fat (<10–130 \mu g/kg). Residues were measured in the subcutaneous fat (<20–25 \mu g/kg) in only one study, but in this study they were higher than in either omental or perirenal fat. Surprisingly, in view of the radiometric data, residues were not measured in the subcutaneous fat at the site of application in the studies using the pour-on formulations. Residues were measured in the whole milk of ewes after dipping once in a 0.015% formulation. The mean residue levels were 13, 10, 9, 7 and 7 \mu g/l, at 1, 3, 7, 10 and 15 days after dipping, respectively. About 5.8% of the milk was butterfat, and if all the residues were present in this, the maximum concentration in the fat was 206 \mu g/kg on day 1; a high value of 143 \mu g/kg was also seen on day 7. The results were not corrected for recoveries, which were in the range 70–85%.

Residues were measured in hens over a 14-day period following administration of either 10 or 20 mg of cypermethrin as a spray. Residues were at or below the quantification limit (10 \mu g/kg) in muscle, liver, kidney and eggs, but were present in fat (25–140 \mu g/kg) and skin (80–1300 \mu g/kg).

In the studies submitted for evaluation, there were low concentrations of bound residues, and >90% of the bound residues could be chemically released and were shown to be metabolites.

\textit{\alpha-Cypermethrin.} A residue-depletion study was carried out in four cows using the radiolabelled drug at the recommended dose as a pour-on preparation. Residues were measured at 7, 14, 28 and 35 days after dosing.

The total residues (measured as radioactivity) in the edible tissues were mostly below the quantification limit (10–30 \mu g/kg), except in the subcutaneous fat of one cow slaughtered at 35 days after dosing, where the level was at the quantification limit (30 \mu g/kg). Perirenal and subcutaneous fat samples from the cows slaughtered at 7 and 14 days after treatment were also analysed by GC with a quantification limit of 10 \mu g/kg, but only trace amounts were found. The levels in perirenal and subcutaneous fat were <10 and 10 \mu g/kg, respectively, at 7 days and 20 \mu g/kg (both tissues) at 14 days. These figures are not significantly different from the estimates of total radioactivity. Thus, the highest level of residues observed in this study was about 30 \mu g/kg in subcutaneous fat. In the milk samples following the pour-on application, mean levels of total radioactivity reached a peak of 7 \mu g/l (range <1–12 \mu g/l) on day 2 and fell to the quantification limit of 1 \mu g/l by day 7.

Residues expressed as \alpha-cypermethrin were measured in cattle, sheep and poultry after the topical application of unlabelled \alpha-
cypermethrin. The results submitted were not corrected for recovery. After application of the pour-on preparation, no residues were detectable in the muscle or liver of young cattle and were <30μg/kg in kidney over the 14-day post-dosing period. In two of the studies there was evidence of persistence of residues in both subcutaneous fat and perirenal fat of calves. The residues in perirenal fat were 60–150μg/kg (mean 90μg/kg) at 14 days after treatment, but then declined to about 10–20μg/kg by day 28, while residues in subcutaneous fat were 10–20μg/kg by day 14.

Residues in bovine milk reached peak values between days 2 and 5 after the pour-on treatment and were below the quantification limit by day 21.

Sheep which were dipped in a preparation containing α-cypermethrin had higher residues in the fat, wool and skin than those which received the pour-on treatment. In one study with the pour-on treatment, residues in fat were not detectable within 7 days of dosing, but in sheep which were dipped the residues were 40μg/kg in fat at 7 and 14 days after dosing. High residues were found in the skin for at least 2 weeks after both treatments.

In another study in sheep which were treated with a pour-on preparation, the residues were 10μg/kg (maximum 18μg/kg) and 6μg/kg (maximum 19μg/kg) in the perirenal and omental fat, respectively, at day 7 after dosing.

In a study in poultry, hens were treated with an α-cypermethrin spray, and residues in eggs measured over a 14-day period. No α-cypermethrin was found in the albumen, but residues in the yolk were 12μg/kg (range <5–45μg/kg) at day 2, 26μg/kg (6–43μg/kg) at day 5, 19μg/kg (<5–43μg/kg) at day 10, and 16μg/kg (5–47μg/kg) at day 14.

Analytical methods

Cypermethrin. Two detailed analytical methods were submitted, one for cypermethrin (quantification limit 10μg/kg) in tissues and one for cypermethrin (detection limit 2μg/l) in milk. Confirmatory methods using GC–MS were described in the papers submitted. The analytical methods were submitted without adequate validation data. Evidence is required of the quantification and detection limits of the methods.

α-Cypermethrin. Suitable methods for the specific analysis of α-cypermethrin in milk and edible tissues were submitted, namely GC with electron-capture detection followed by confirmation with GC–MS. The quantification limits claimed were 1μg/l for milk and 10μg/kg for tissues. Recoveries were between 80% and 105%.
On the basis of the available data, the only possible option for a marker substance is the parent compound, which accounts for the majority of the total residues in muscle, milk, and fat. On the basis of a study in one cow treated orally, the proportion of the total residues accounted for by  \( \alpha \)-cypermethrin in liver and kidney was tentatively estimated at 16% and 20%, respectively. Thus, only limited information was available on the proportion of the total residues accounted for by  \( \alpha \)-cypermethrin in these tissues and in skin. However, the total residues were below the quantification limit in liver and kidney, so that the sensitivity of the analytical methods could be considered in recommending an MRL for these tissues. Fat is the recommended target tissue. Whole milk or milk fat is suitable for monitoring residues in milk, and egg yolk for monitoring residues in eggs.

**Maximum Residue Limits**

**Cypermethrin.** In recommending MRLs for cypermethrin, the Committee took into account the following factors:

- The ADI is 0–50\( \mu g \) per kg of body weight, which is equivalent to a maximum ADI of 3000\( \mu g \) for a 60-kg person. The ADI is the same as that established by the 1981 Joint FAO/WHO Meeting on Pesticide Residues (9).
- The parent drug is the marker residue.
- Fat is the target tissue, but muscle, liver and kidney should also be considered. Milk and eggs are also recommended for monitoring residues.
- The metabolism and residue-depletion studies using the radiolabelled drug were not adequate, and therefore very conservative estimates of the proportion of the total residues accounted for by the marker compound in all target species were proposed. The estimates were 30% for muscle, 10% for liver, 5% for kidney, 60% for fat, 80% for milk and 30% for eggs.
- There was adequate information on residues from the residue-depletion studies using the unlabelled drug in the recommended formulations.
- Analytical methods were available; however, evidence of adequate validation was needed.

The Committee recommended temporary MRLs for cypermethrin of 200\( \mu g \)/kg for muscle, liver and kidney and 1000\( \mu g \)/kg for fat, expressed as parent drug, in cattle, sheep and chickens. The Committee also recommended temporary MRLs of 50\( \mu g \)/l and 100\( \mu g \)/kg for whole milk (cows’) and eggs (chickens), respectively.

From these values for the MRLs, the maximum theoretical intake would be 810\( \mu g \) per day (Table 6). This would be compatible with a
Table 6
Theoretical maximum daily intake of cypermethrin residues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Recommended MRL (µg/kg)⁶</th>
<th>Estimate of total residues (µg/kg)</th>
<th>Theoretical maximum daily intake⁸ (µg cypermethrin equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>200</td>
<td>666⁵</td>
<td>200</td>
</tr>
<tr>
<td>Liver</td>
<td>200</td>
<td>2000⁵</td>
<td>200</td>
</tr>
<tr>
<td>Kidney</td>
<td>200</td>
<td>4000⁶</td>
<td>200</td>
</tr>
<tr>
<td>Fat</td>
<td>1000</td>
<td>1666⁵</td>
<td>83</td>
</tr>
<tr>
<td>Milk</td>
<td>50⁹</td>
<td>63³,⁸</td>
<td>94</td>
</tr>
<tr>
<td>Eggs</td>
<td>100</td>
<td>333⁵</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>810</td>
</tr>
</tbody>
</table>

⁶ Expressed as parent drug.
⁷ Based on a daily intake of 0.5kg of meat made up of 500g of muscle, 100g of liver, 50g of kidney and 50g of fat, 1.61g of milk and 10g of eggs.
⁸ The marker residue accounted for 30% of the total residues in muscle and eggs.
⁹ The marker residue accounted for 10% of the total residues in liver.
¹⁰ The marker residue accounted for 5% of the total residues in kidney.
¹¹ The marker residue accounted for 10% of the total residues in fat.
¹² Expressed in µg/.
¹³ The marker residue accounted for 80% of the total residues in milk.

maximum ADI of 3000µg for a 60-kg person. The Committee noted that the theoretical maximum intake of cypermethrin calculated using the daily food intake values established by the Joint FAO/WHO Meeting on Pesticide Residues is approximately 300µg. The MRLs therefore accommodate the recommended uses of this compound both as a veterinary drug and as a pesticide.

The following information is required for evaluation in 2000:

1. The results of residue-depletion studies using radiolabelled cypermethrin that extend beyond the recommended withdrawal times for the drug in its topical formulation. The depletion of the total residues and of the parent drug should be determined.
2. Evidence that interconversion of isomeric forms does not occur during metabolism in the target species.
3. Further information on the validation of the analytical methods, particularly data on the derivation of the detection and quantification limits.

α-Cypermethrin. In recommending MRLs for α-cypermethrin, the Committee took into account the following factors:

- The ADI is 0–20µg per kg of body weight per day, which is equivalent to a maximum ADI of 1200µg for a 60-kg person.
- The parent drug is the marker residue.
• Fat is the target tissue, but muscle, liver and kidney should also be considered. Whole milk or milk fat is suitable for monitoring residues in milk, and egg yolk for monitoring residues in eggs.
• The metabolism of the two isomers which form α-cypermethrin is similar to that of the other six isomers in cypermethrin.
• The metabolism and residue-depletion studies using the radiolabelled drug were not adequate, and therefore very conservative estimates of the proportion of the total residues accounted for by the marker compound in all target species were proposed. The percentages proposed for the estimation in individual tissues of total residues from the parent drug were 30% for muscle, 10% for liver, 5% for kidney and 60% for fat. In milk and eggs, α-cypermethrin was estimated to account for 80% and 30% of the total residues, respectively.
• There was adequate information on residues from the residue-depletion studies using the unlabelled drug in the recommended formulations.
• Analytical methods were available; however, evidence of validation was needed.

The Committee recommended temporary MRLs for α-cypermethrin of 100µg/kg for muscle, liver and kidney and 500µg/kg for fat, expressed as parent drug, in cattle, sheep and chickens. It also recommended temporary MRLs of 25µg/l for whole milk and 50µg/kg for eggs, expressed as parent drug, in cattle and chickens, respectively.

From these values for the MRLs, the maximum theoretical intake would be 406µg per day (Table 7). This would be compatible with the ADI and the recommended use of this compound as a veterinary drug.

The following information is required for evaluation in 2000:

1. The results of residue-depletion studies using radiolabelled α-cypermethrin in sheep and chickens that extend beyond the recommended withdrawal times for the drug in its topical formulation. The depletion of the total residues and of the parent drug should be determined.
2. The residue-depletion study using radiolabelled α-cypermethrin in cattle that was submitted should be reassessed to determine the depletion of the total residues and of the parent drug.
3. Evidence that interconversion of isomeric forms does not occur during metabolism in the target species.
Table 7
Theoretical maximum daily intake of α-cypermethrin 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 α-cypermethrin equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>100</td>
<td>333(^c)</td>
<td>100</td>
</tr>
<tr>
<td>Liver</td>
<td>100</td>
<td>1000(^d)</td>
<td>100</td>
</tr>
<tr>
<td>Kidney</td>
<td>100</td>
<td>2000(^e)</td>
<td>100</td>
</tr>
<tr>
<td>Fat</td>
<td>500</td>
<td>833(^f)</td>
<td>42</td>
</tr>
<tr>
<td>Milk</td>
<td>25(^g)</td>
<td>31(^h)</td>
<td>47</td>
</tr>
<tr>
<td>Eggs</td>
<td>50</td>
<td>166(^i)</td>
<td>17</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>406</strong></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, 50 g of kidney and 50 g of fat, 1.5 l of milk and 100 g of eggs.  
\(^c\) The marker residue accounted for 30% of the total residues in muscle and eggs.  
\(^d\) The marker residue accounted for 10% of the total residues in liver.  
\(^e\) The marker residue accounted for 5% of the total residues in kidney.  
\(^f\) The marker residue accounted for 80% of the total residues in fat.  
\(^g\) Expressed in µg/l.  
\(^h\) The marker residue accounted for 80% of the total residues in milk.

4. Further information on the validation of the analytical methods, particularly data on the derivation of the detection and quantification limits.

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.

**Acknowledgement**

The Expert Committee wishes to thank Dr P. Jenkins, International Programme on Chemical Safety, WHO, Geneva, Switzerland, for his assistance in the preparation of the report.

**References**


Annex 1

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


63. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 18, 1983.

64. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 28, 1983.


82. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41, 1988 (out of print).


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


121. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/8, 1996.


Annex 2
Recommendations on compounds on the agenda, and further toxicological studies and other information required

Adrenoceptor agonists

**Clenbuterol**

ADI: 0–0.004 µg per kg of body weight.

Residue definition: Clenbuterol.

<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>0.2</td>
<td>0.6</td>
<td>0.6</td>
<td>0.2</td>
<td>0.05</td>
</tr>
<tr>
<td>Horses</td>
<td>0.2</td>
<td>0.6</td>
<td>0.6</td>
<td>0.2</td>
<td>—</td>
</tr>
</tbody>
</table>

* Expressed in µg/l.

**Xylazine**

ADI: The Committee was unable to establish an ADI for xylazine because it concluded that the metabolite 2,6-xylidine was genotoxic and carcinogenic.

Residue definition: The Committee was unable to establish MRLs for xylazine because of the lack of information on metabolism and residue depletion in edible tissues.

Before reviewing this compound further, the Committee requires the following information:

- Sufficient data on the metabolism of xylazine in target species for it to identify a suitable marker residue and target tissue(s).
- A suitable analytical method for measuring the marker residue in the target tissue(s).
- Additional data on the residue depletion of xylazine and its metabolites in target species, including evidence to show whether 2,6-xylidine is present at the recommended withdrawal times.
Anthelmintic agents

Abamectin

ADI: 0–1 µg per kg of body weight (established by the 1995 Joint FAO/WHO Meeting on Pesticide Residues (FAO Plant Production and Protection Paper, No. 133, 1996)).

Residue definition: Avermectin B₁₉.

<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>—³</td>
</tr>
</tbody>
</table>

³ The Committee concluded that there was no need for an MRL for bovine muscle, since abamectin residues are below detectable levels at the recommended withdrawal time.
² Abamectin is intended only for use in beef cattle.

Moxidectin

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

Residue definition: Moxidectin (MRLs, except for sheep muscle, were established at the forty-fifth meeting of the Committee (WHO Technical Report Series, No. 864, 1996)).

<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>20⁴</td>
</tr>
<tr>
<td>Sheep</td>
<td>50⁶</td>
</tr>
<tr>
<td>Deer</td>
<td>20⁴</td>
</tr>
</tbody>
</table>

⁴ At the forty-fifth meeting (WHO Technical Report Series, No. 864, 1996), the Committee noted the very high concentrations and great variation in the level of residues at the injection site in cattle muscle over a 49-day period after dosing.
⁵ 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.
⁶ Established at the present meeting.
⁷ Temporary MRLs, pending the receipt of further information on the marker residue in the edible tissues. This information is required for evaluation in 1998 (WHO Technical Report Series, No. 864, 1996).

Antimicrobial agents

Chlortetracycline, oxytetracycline and tetracycline

ADI: 0–3 µg per kg of body weight (established at the forty-fifth meeting of the Committee (WHO Technical Report Series, No. 864, 1996)).
Residue definition: Chlortetracycline and tetracycline (MRLs, except for sheep muscle, were recommended at the forty-fifth meeting of the Committee (WHO Technical Report Series, No. 864, 1996)).

Oxytetracycline (MRLs were recommended at the thirty-sixth meeting of the Committee (WHO Technical Report Series, No. 799, 1990)).

<table>
<thead>
<tr>
<th>Species</th>
<th>Recommended MRLs (µg/kg)</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
<th>Milk$^d$</th>
<th>Eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>100</td>
<td>300</td>
<td>600</td>
<td>—</td>
<td>—</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>Pigs</td>
<td>100</td>
<td>300</td>
<td>600</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Sheep</td>
<td>100</td>
<td>300</td>
<td>600</td>
<td>—</td>
<td>—</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>Poultry</td>
<td>100</td>
<td>300</td>
<td>600</td>
<td>—</td>
<td>—</td>
<td>200</td>
<td>—</td>
</tr>
<tr>
<td>Fish</td>
<td>100$^c$</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Giant tiger prawn (Penaeus monodon)</td>
<td>100$^c$</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

$^a$ Expressed in µg/l.
$^b$ 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.
$^c$ Applies only to oxytetracycline.

**Neomycin**

**ADI:** 0–60µg per kg of body weight.

Residue definition: Neomycin (MRLs, except for kidney in cattle, pigs, sheep, goats, chickens, ducks and turkeys, were recommended at the forty-third meeting of the Committee (WHO Technical Report Series, No. 855, 1995)).

<table>
<thead>
<tr>
<th>Species</th>
<th>Recommended MRLs (µg/kg)</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
<th>Milk$^d$</th>
<th>Eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>500</td>
<td>500</td>
<td>10 000</td>
<td>500</td>
<td>500</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Pigs</td>
<td>500</td>
<td>500</td>
<td>10 000</td>
<td>500</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Sheep</td>
<td>500</td>
<td>500</td>
<td>10 000</td>
<td>500</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Goats</td>
<td>500</td>
<td>500</td>
<td>10 000</td>
<td>500</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Turkeys</td>
<td>500</td>
<td>500</td>
<td>10 000</td>
<td>500</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ducks</td>
<td>500</td>
<td>500</td>
<td>10 000</td>
<td>500</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Chickens</td>
<td>500</td>
<td>500</td>
<td>10 000</td>
<td>500</td>
<td>—</td>
<td>500</td>
<td>—</td>
</tr>
</tbody>
</table>

$^a$ Expressed in µg/l.
**Spiramycin**

**ADI:** 0–50 μg per kg of body weight (established at the forty-third meeting of the Committee (WHO Technical Report Series, No. 855, 1995)).

**Residue definition:** Sum of the concentrations of spiramycin and neospiramycin for cattle and chickens (MRLs for bovine fat and cows’ milk, and chicken muscle, kidney and fat were recommended at the forty-third meeting of the Committee (WHO Technical Report Series, No. 855, 1995)).

Spiramycin equivalents for pigs (MRLs for muscle, liver and kidney were recommended at the forty-third meeting of the Committee (WHO Technical Report Series, No. 855, 1995)).

<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>200a</td>
</tr>
<tr>
<td>Pigs</td>
<td>200a</td>
</tr>
<tr>
<td>Chickens</td>
<td>200a</td>
</tr>
</tbody>
</table>

* Expressed as μg/l.

+ Expressed as the sum of the concentrations of spiramycin and neospiramycin.

+ Expressed as spiramycin equivalents.

**Thiamphenicol**

**ADI:** 0–6 μg per kg of body weight (designated as temporary because only a summary report of the carcinogenicity study in rats was available).

**Residue definition:** Thiamphenicol.

<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>40a</td>
</tr>
<tr>
<td>Chickens</td>
<td>40a</td>
</tr>
</tbody>
</table>

* Temporary MRLs.

+ No MRLs were proposed for cows’ milk, as no data were submitted on total residues in milk.

+ MRLs were not recommended for eggs because of the unacceptably high thiamphenicol residues.
The following information is required for evaluation in 1999:

1. Detailed reports on the carcinogenicity study in rats for which the summary report was available at the present meeting and on the range-finding study used to establish dose levels in that study.
2. Residue-depletion studies with radiolabelled and unlabelled thiamphenicol for the identification of the marker residue and target tissues in poultry, pigs and young (non-ruminant) calves.

**Tilmicosin**

**ADI:** 0–40µg per kg of body weight.

**Residue definition:** Tilmicosin.

<table>
<thead>
<tr>
<th>Species</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
<th>Milk&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>100</td>
<td>1000</td>
<td>300</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>Pigs</td>
<td>100</td>
<td>1500</td>
<td>1000</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>Sheep</td>
<td>100</td>
<td>1000</td>
<td>300</td>
<td>100</td>
<td>50&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Expressed as µg/l.
<sup>b</sup> Temporary MRLs pending the receipt of the results of a study using radiolabelled tilmicosin in lactating sheep aimed at determining the relationship between total residues and the parent drug in milk.

The results of a study in lactating sheep with radiolabelled tilmicosin aimed at determining the relationship between total residues and the parent drug in milk are required for evaluation in 1999.

**Insecticides**

**Cypermethrin**

**ADI:** 0–50µg per kg of body weight.

**Residue definition:** Cypermethrin.

<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>200&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sheep</td>
<td>200&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chickens</td>
<td>200&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Expressed in µg/l.
<sup>b</sup> Temporary MRLs.

The following information is required for evaluation in 2000:

1. The results of residue-depletion studies using radiolabelled cypermethrin that extend beyond the recommended withdrawal
times for the drug in its topical formulation. The depletion of the total residues and of the parent drug should be determined.

2. Evidence that interconversion of isomeric forms does not occur during metabolism in the target species.

3. Further information on the validation of the analytical methods, particularly data on the derivation of the detection and quantification limits.

**α-Cypermethrin**

ADI: 0–20μg per kg of body weight.

Residue definition: α-Cypermethrin.

<table>
<thead>
<tr>
<th>Species</th>
<th>Recommended MRLs (μg/kg)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
<td>Liver</td>
</tr>
<tr>
<td>Cattle</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sheep</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chickens</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Expressed in μg/l.

<sup>b</sup> Temporary MRLs.

The following information is required for evaluation in 2000:

1. The results of residue-depletion studies using radiolabelled α-cypermethrin in sheep and chickens that extend beyond the recommended withdrawal times for the drug in its topical formulation. The depletion of the total residues and of the parent drug should be determined.

2. The residue-depletion study using radiolabelled α-cypermethrin in cattle that was submitted should be reassessed to determine the depletion of the total residues and of the parent drug.

3. Evidence that interconversion of the isomeric forms does not occur during metabolism in the target species.

4. Further information on the validation of the analytical methods, particularly data on the derivation of the detection and quantification limits.
Annex 3
Assessing the effects of antimicrobial drug residues in food on the human intestinal microflora

1. Introduction

In recent years, questions have been raised concerning the consumption of low levels of antimicrobial residues in foods from food-producing animals and the effect of these residues on the indigenous human intestinal microflora. Guidelines from the CVMP Working Party on the Safety of Residues of the Commission of the European Communities and the Food and Drug Administration in the United States indicate that the evaluation of the microbiological risks from antimicrobial residues must take into consideration their potentially harmful effects on the human gut flora (2–5). The following questions have been raised:

(a) What effects do low levels of antimicrobial residues have on the microbial ecology of the human gastrointestinal tract?
(b) What concentration of an antimicrobial residue would have “no adverse effect” on the human intestinal microflora?
(c) What proportion of the dose of the antimicrobial is available to the bacteria in the lower part of the gastrointestinal tract?
(d) What experimental approaches should be used to measure potential microbiological effects?
(e) What magnitude of change in test microbiological end-point(s) is indicative of normal variation?
(f) What magnitude of change in test microbiological end-point(s) is indicative of a potential impact on individual health?

The microflora of the human gastrointestinal tract forms an extremely complex, yet relatively stable, ecological community, with over $10^{14}$ bacterial cells per gram of content and more than 400 bacterial species (6–8). Approximately 90% of these species are obligate anaerobes of 30 different species. The predominant genera are Bacteroides, Eubacterium, Clostridium, Fusobacterium, Ruminococcus, Peptococcus and Peptostreptococcus (see Table 1). Although there may be large variations between individuals in the proportions of the major species present depending on the diet, the population sizes of different species from the same individual are stable (8, 9). The intestinal microflora is an essential component of human physiology.

---

1 Originally based on a paper first considered at the forty-fifth meeting of the Committee (1). The final text represents the Committee’s views and conclusions.
Table 1

Predominant bacterial species isolated from the gastrointestinal tract of healthy adults

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>Genus</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroides</td>
<td>B. vulgatus</td>
<td>Peptostreptococcus</td>
<td>P. anaerobius</td>
</tr>
<tr>
<td></td>
<td>B. uniformis</td>
<td></td>
<td>P. productus</td>
</tr>
<tr>
<td></td>
<td>B. stercoris</td>
<td></td>
<td>P. parvulus</td>
</tr>
<tr>
<td></td>
<td>B. fragilis</td>
<td></td>
<td>P. micros</td>
</tr>
<tr>
<td></td>
<td>B. ovatus</td>
<td></td>
<td>P. prevotii</td>
</tr>
<tr>
<td></td>
<td>B. caceae</td>
<td>Ruminococcus</td>
<td>R. bromii</td>
</tr>
<tr>
<td></td>
<td>B. distasonis</td>
<td></td>
<td>R. obeum</td>
</tr>
<tr>
<td></td>
<td>B. thetactomicron</td>
<td></td>
<td>R. gnarus</td>
</tr>
<tr>
<td></td>
<td>B. capillosus</td>
<td></td>
<td>R. callidus</td>
</tr>
<tr>
<td></td>
<td>B. merdae</td>
<td></td>
<td>R. torques</td>
</tr>
<tr>
<td>Fusobacterium</td>
<td>F. prausnitzii</td>
<td></td>
<td>R. albus</td>
</tr>
<tr>
<td></td>
<td>F. russii</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilidobacterium</td>
<td>B. adolescentis</td>
<td>Enterococcus</td>
<td>E. faecalis</td>
</tr>
<tr>
<td></td>
<td>B. longum</td>
<td></td>
<td>E. siraem</td>
</tr>
<tr>
<td></td>
<td>B. catenulatum</td>
<td>Lactobacillus</td>
<td>L. acidophilus</td>
</tr>
<tr>
<td></td>
<td>B. infantis</td>
<td></td>
<td>L. fermentum</td>
</tr>
<tr>
<td>Eubacterium</td>
<td>E. aerofaciens</td>
<td>Escherichia</td>
<td>E. coli</td>
</tr>
<tr>
<td></td>
<td>E. rectale</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>E. biforme</td>
<td>Propionibacterium</td>
<td>P. acnes</td>
</tr>
<tr>
<td></td>
<td>E. eligens</td>
<td>Methanobrevibacter</td>
<td>M. smithii</td>
</tr>
<tr>
<td></td>
<td>E. lentum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium</td>
<td>C. perfringens</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. butyricum</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. ramosum</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. indolis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Adapted from references 6–11.

because it acts as a barrier against colonization of the gastrointestinal tract by pathogenic bacteria. It also plays important roles in the digestion of food and in the metabolism of drugs, xenobiotics and nutrients (12).

The major sources of human exposure to antimicrobial drugs are listed in Table 2. For those antimicrobial drugs that reach the colon in sufficiently high concentrations of the active form, there is the potential for them to exert detrimental effects on the ecological balance of the microflora. Repeated exposure to therapeutic doses of antimicrobials may perturb the normal human intestinal microflora by changing the population density, altering the activity of enzymes involved in the metabolism of endogenous and exogenous substances, and impairing resistance to colonization which may increase susceptibility to infection by enteric pathogens such as Salmonella spp., Shigella spp. and Escherichia coli (10, 13–16). Antimicrobial compounds also exert a selective pressure on the indigenous microflora, which could increase the potential for selecting resistance to antibiotics in the dominant

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Table 2
Sources of human exposure to antimicrobial drugs

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Source of human exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Therapeutic</td>
<td>Clinical exposure/antimicrobial therapy</td>
</tr>
<tr>
<td>Subtherapeutic</td>
<td>Antimicrobial residues in food</td>
</tr>
<tr>
<td></td>
<td>Ingestion of antimicrobial-resistant microorganisms in food</td>
</tr>
<tr>
<td></td>
<td>Environmental exposure (e.g. to faecal wastes or aquaculture effluent)</td>
</tr>
<tr>
<td></td>
<td>Endogenous antimicrobials in food</td>
</tr>
</tbody>
</table>

intestinal microflora through increased acquisition of antibiotic-resistant plasmids (17). In addition, therapeutic concentrations could potentially enhance the development of acquired resistance in enteric bacteria (18). However, little is known about adverse effects on the intestinal microflora resulting from exposure to low concentrations (of the order of mg/kg) of antimicrobial residues in food. Therefore, it is important to determine the concentrations of antimicrobials that produce “no adverse effects” on the human intestinal microflora.

2. Methods for assessing the effects of antimicrobial drug residues on the human intestinal microflora

A variety of in vitro and in vivo methods for studying the effects of antimicrobial drug residues on the intestinal microflora have been used for assessing the potential risks of these compounds to humans, as outlined in Table 3. Each method has its advantages and disadvantages, and there is much debate as to their relevance to the assessment of the microbiological risk to humans (19–23).

2.1 In vitro models

Several in vitro models have been developed for assessing the effects of diet, food additives and drugs on the human intestinal microflora. These models are based on three approaches: batch culture, MIC assays, and continuous and semi-continuous culture systems. The advantages and disadvantages of the various methods have recently been reviewed (20) and are summarized in Table 3. Data from in vitro MIC studies have been used routinely for estimating the effects of antimicrobial agents on the human gastrointestinal microflora (section 3.1). Other in vitro methods, such as continuous and semi-continuous culture systems, which mimic the human intestinal ecosystem, can provide data on the development of antibiotic resistance in the predominant intestinal microflora, resistance to colonization by pathogenic microorganisms and alterations in the metabolic activity of the microflora (20) (see Table 3). These models simulate the
<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Relevance to human risk assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vitro</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batch culture</td>
<td>Rapid method of screening faecal specimens or pure cultures. Relatively inexpensive, simple and convenient to perform</td>
<td>Does not simulate the reactions of the microflora in vivo. Does not take into account absorption, excretion and distribution in humans or colonization barrier effects</td>
<td>Low to moderate</td>
</tr>
<tr>
<td>Determination of minimum inhibitory concentration (MIC)</td>
<td>Relatively simple method of screening a number of pure cultures representative of the human gastrointestinal tract. Relatively inexpensive, fast and easy to perform</td>
<td>Not representative of the ecological system in the human gastrointestinal tract. However, new methods are being developed to mimic conditions in the human intestine</td>
<td>Moderate</td>
</tr>
<tr>
<td>Continuous and semi-continuous culture systems</td>
<td>Mimic microflora interactions in the human large intestine. Functional end-points, such as hydrolytic and reductive enzyme reactions, gas production, and volatile and non-volatile fatty acid formation can be determined. Selective pressure due to antimicrobial exposure to the microflora can be measured. Resistance to colonization by pathogenic microorganisms can be determined</td>
<td>More complex than MIC determinations. Not validated to any significant extent</td>
<td>Moderate</td>
</tr>
<tr>
<td><strong>In vivo</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Studies on conventional laboratory animals</td>
<td>Relatively simple to perform and can control a wide variety of dietary and environmental parameters. A wide range of parameters, including metabolic interactions between different species of the microflora, resistance to colonization by pathogenic microorganisms, selective pressure and functional end-points can be determined</td>
<td>The intestinal microflora of rodents is significantly different from that of humans. Validation studies are limited</td>
<td>Low</td>
</tr>
</tbody>
</table>
fermentation process and the microbial community in the colon. Changes in the microflora are usually measured either by selective culture and identification of the predominant species present and determining the number and proportion of enterobacteria present, which is an indication of the degree of resistance to colonization, or by measuring the biochemical activities of the bacterial enzymes in human faecal samples.

The isolation and identification of bacteria from faecal samples is time-consuming and can be imprecise because the taxonomy of the intestinal microflora is not fully understood. However, rapid molecular techniques for determining the predominant intestinal microflora of animals and humans have been reported (24). In general, studies on the human intestinal flora have been restricted to those bacterial species normally found in faecal samples, which are from the terminal colon and rectum. More complex procedures are required to isolate the flora of higher sections of the gastrointestinal tract. An understanding is slowly being developed of the composition of the mucosal flora of the large bowel from endoscopic studies (7). The mucosa-associated flora is different from that of the gut lumen, because oxygen diffusion from the mucosa is significant and is capable of inhibiting the strictly anaerobic flora, thereby allowing the proliferation of aerobic and microaerophilic flora. Instead of measuring absolute numbers of intestinal microflora directly, the biochemical
activities of the intestinal microflora can be monitored to detect population changes during exposure to xenobiotics. These biochemical end-points may include concentrations of short-chain fatty acids, activities of certain enzymes (β-glucuronidase, β-glucosidase, nitroreductase, nitrate reductase, azoreductase, 7-α-dehydroxylase), methane production, sulfate reduction, analysis of total cellular fatty acid, and bile acid metabolism (20). Measurements of biochemical activities may be useful as indicators of the attainment of a steady state and the stability of the intestinal microflora population. However, both the interpretation of a change in metabolic activity and what effect the magnitude of the change has on the intestinal microflora and human health are still uncertain.

2.2 Animal models

The best way of determining the effect of low-level doses of antimicrobials on human intestinal microflora would be by means of studies in human volunteers. However, since studies of this type may be unethical and there are technical difficulties (see Table 3), other in vitro and in vivo methods have been proposed (19, 21, 23). In vivo studies using human flora associated (HFA) rats and mice (see below), although expensive to conduct, provide data that are more relevant to humans (25–28).

The HFA rodent model has been used by several laboratories to test the effects of therapeutic doses of antimicrobials in vivo, since the intestinal microflora of rodents is significantly different from that of humans. Although the HFA rodent model has high relevance in determining the effects of low levels of antimicrobials on human microflora, it still needs to be validated before guidelines are established by regulatory authorities. The major advantage of this experimental model is that it has greater relevance to humans than studies on conventional rodents, since human microflora in the form of faecal suspensions is fed to germ-free animals and subsequent colonization is with a well defined flora, very similar to that in humans, with similar enzymatic activities to those detected in the microflora from human donors. In addition, the microflora is similar in all the experimental animals tested before antimicrobial treatment, and contains a low background level of antibiotic-resistant organisms. The major disadvantages are that a germ-free animal facility is expensive, the results may depend on the microflora of the human donors, the physiology of rodents is different from that of humans, and rodents and humans metabolize many compounds differently (19, 20, 23).

It should be noted that few in vitro continuous culture systems and in vivo studies in experimental animals have been validated. More
studies should be carried out to determine the variability and reproducibility of any protocols used to provide data on the effect of low levels of antibiotics on human intestinal microflora and their relevance to human exposure.

3. **Approaches to assessing the effects of antimicrobial compounds on the human intestinal microflora**

3.1 **Joint FAO/WHO Expert Committee on Food Additives**

At its thirty-eighth, forty-second, forty-third and forty-fifth meetings (1, 2, 29, 30), the Joint FAO/WHO Expert Committee on Food Additives assessed the susceptibility of human intestinal microflora to a number of antimicrobial agents. In many cases, an ADI based on antimicrobial activity was determined, using an equation that was developed at the thirty-eighth meeting in 1991 (2):

\[
\text{Upper limit of temporary ADI (µg per kg of body weight)} = \frac{\text{Concentration without effect} \times \text{Daily faecal bolus (150 g) on human gut flora (µg/ml)}}{\text{Fraction of oral dose} \times \text{Safety factor} \times \text{Weight of human bioavailable (60 kg)}}
\]

This equation is both convenient and simple to use, but has not been validated for relevance to the human intestine *in vivo* (Table 3). A number of questions have arisen, however, about the values that should be used for each of the parameters. The equation is based on a number of assumptions, as described below.

*Concentration without effect on the human gut flora*

The concentration without microbiological effect on the human gut flora has been based on MIC data. MIC assays are quick, relatively inexpensive, and provide data showing very low inhibitory concentrations for bacterial cultures representative of the human intestinal tract (see Table 1). The use of this information in the formula may result in an excessively conservative estimate of the ADI for the following reasons:

- The MIC test was designed to provide a reproducible assay of *in vitro* activity of antimicrobials against bacterial pathogens, not intestinal microflora. The reliability of the standardized MIC test for a number of pathogenic anaerobic bacteria has recently been questioned (31, 32). Many of these pathogens are more readily cultivated than intestinal microflora anaerobes. This makes the usefulness of the standardized MIC test for anaerobic bacteria
suspect for many organisms which do not achieve adequate or vigorous growth on standard MIC test media.

- It is likely that the interactions between the antimicrobial agent and the individual strains in the MIC assay are vastly different from those that occur in the complex mixture of high-density microbial populations in the gastrointestinal tract.
- The ecological and metabolic interactions among the major species are not measured in these assays.
- The inoculum density in conventional MIC determinations is low in comparison with the situation in the intestine.
- MIC data do not fully mimic exposure to antimicrobial residues because MIC assays measure only acute effects. Concerns about the safety of food containing residues of veterinary drugs usually focus on chronic low-level exposure.
- Changes in resistance to colonization and to antibiotics in the normal intestinal microflora cannot be determined by means of MIC tests.

If MIC data are to be used for estimating the microbiological risk of antimicrobial agents, it is important that the microorganisms chosen for evaluation be representative of the human intestinal microflora and that techniques recommended by the National Committee for Clinical Laboratory Standards (33, 34), with modifications to mimic conditions in the human gastrointestinal tract, be used for making the MIC determinations.

**Daily faecal bolus**

The value of the daily faecal bolus used in the equation is questionable. A human faecal weight of 150g underestimates the colonic volume of a 60-kg person. The Committee therefore concluded that it is more appropriate to replace it by an estimate of the mass of the colonic contents (MCC) (220 g) (35).

**Fraction of oral dose bioavailable**

This should be based on the fraction of the oral dose that would be expected to reach the colon and affect the microflora, i.e. the fraction available to microflora in the colon. In addition, binding may occur between an antibiotic and the contents of the human intestine, which will reduce the impact of the antibiotic residue on the intestinal microflora. Enterohepatic circulation of the antimicrobial agent should also be taken into consideration as a source of antimicrobial exposure.
Weight of human
A value of 60 kg, which represents the average body weight of an adult, has been used in the past, but its relevance to the calculation is questionable. However, this factor has been retained since this value conforms with the approach adopted for the calculation of an ADI based on toxicological data and permits direct comparison with it.

Safety factor
The Committee considered that the safety factor should be clarified, because without a defined safety factor inconsistencies may arise in the calculation of the ADI. A safety factor is usually applied in the process of estimating ADIs based on the results of conventional toxicological studies. In general, it is assumed that humans will be more sensitive than the test species, and the value of the safety factor is selected accordingly. As outlined earlier, in vitro MIC tests result in low inhibitory concentrations. These methods tend to use single bacterial cultures of around 10^6–10^8 cells per ml and are carried out under optimum conditions in an artificial medium. The test organisms are, in general, likely to be inhibited in vitro at lower concentrations than would be expected to be effective in the gut, given the complex mixture of microorganisms present at around 10^13 cells per gram of the intestinal contents. If data from in vitro MIC tests are to be used in the equation, the appropriateness of a safety factor greater than unity is questionable, since the experimental results are obtained in a system which is probably more sensitive than the human gastrointestinal tract. The incorporation of a safety factor less than unity may be difficult to justify, and a safety factor of 1 could therefore be adopted if extensive MIC data are available for relevant species (Table 1) at high inoculum numbers under strictly anaerobic conditions.

Modification of the equation for calculating an ADI on the basis of in vitro MIC data
Taking into account some of the parameters discussed above, the Committee modified the equation for determining the upper limit of the ADI as follows:

\[ \text{Upper limit of ADI (µg per kg of body weight)} = \frac{\text{MIC}_{50} \text{ (µg/g)} \times \text{Mass of colonic contents (g)}}{\text{Fraction of oral dose} \times \text{Safety factor} \times \text{Body weight (kg)}} \]

where:

\( \text{MIC}_{50} \) = 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 this evaluation, the MIC₉₀ value is the mean MIC₉₀ for the strain(s) of the relevant species tested. Alternatively, the lowest MIC₉₀ for the most sensitive species can be used.

A value of 220 g is used for the mass of the colonic contents (32). The safety factor used to take account of uncertainty about the amount and relevance of MIC data available for review may range from 1 to 10. A value of 1 will be used when extensive relevant microbiological data are available.

3.2 Committee for Veterinary Medicinal Products

The approach adopted by the Committee for Veterinary Medicinal Products (CVMP) (5) of the Commission of the European Communities differs slightly from that of the Joint FAO/WHO Expert Committee on Food Additives. The equation used for determining an ADI based on antimicrobial activity is as follows:

\[
\text{ADI (µg per kg of body weight)} = \frac{(\text{Geometric mean MIC}_{90} [µg/ml] \times \text{CF2})}{(\text{CF1} \times \text{Daily faecal bolus (150 g)})} 
\]

\[
\frac{\text{Fraction of oral dose available} \times \text{Weight of human (60 kg)}}{\text{to microorganisms}}
\]

where:

CF1 = A correction factor to account for the range of MIC₉₀ values, the risk for selection of multi-resistant bacteria, and the induction of resistance to colonization.

CF2 = A correction factor to account for differences between the growth conditions in vitro and those in vivo.

The CVMP considered it to be appropriate to use the geometric mean rather than the arithmetic mean MIC₉₀, since the former is less influenced by values that are much larger or smaller than most values.

The other differences are that the CVMP continues to use the daily faecal bolus (rather than the mass of the colonic contents) and the correction factors used are more discretionary than the safety factor applied by the Joint FAO/WHO Expert Committee on Food Additives. Nevertheless, the results obtained by the two approaches are likely to be similar.
3.3 Food and Drug Administration

The Center for Veterinary Medicine of the Food and Drug Administration in the United States has not adopted either of the approaches discussed in sections 3.1 and 3.2. At present, the Food and Drug Administration does not accept the use of *in vitro* MIC data for establishing a microbiological no-effect level because it does not believe that these data predict the level of drug residues which give rise to the potential public health concerns.

Sponsors are responsible for adequately demonstrating the microbiological activity of their product and for monitoring the appropriate microbiological end-points(s) to establish the antimicrobial no-effect level in an appropriate model.

Before any study is conducted, the antimicrobial agent should be characterized. The chemical structure, antimicrobial drug class and spectrum of antimicrobial activity (defined in terms of potential adverse effects on the intestinal microflora) should be identified. Data from *in vitro* systems or clinical studies in humans will generally be appropriate for these preliminary determinations.

The Food and Drug Administration has identified three categories of antimicrobial agents that in most cases will not require special microbiological testing for human food safety:

- antimicrobial drugs with very low residue levels;
- antimicrobial drug residues with limited antimicrobial activity; and
- antimicrobial drugs for veterinary use which have no adverse effects on the intestinal microflora at the doses approved for the target species (4).

The Food and Drug Administration has suggested that microbiological studies are not necessary when the maximum intake of residues of an antimicrobial agent is at or below 1.5 mg per person per day. This level is based on the supposition that a microbial agent with an MRL of 1 mg/kg or less does not present a microbiological hazard, assuming that the average person consumes 1.5 kg of food per day containing the agent. If the levels of antimicrobial residues in foods are higher than 1.5 mg per person per day, assessments are based on the results of microbiological tests.

The assumption that 1.5 mg per person per day represents a "very low" value in terms of microbiological activity is based on the fact that the effects of antimicrobials on the colonic bacteria are minimized by the large number present and their slow growth rate. Moreover, all studies on antimicrobials performed to date support the view that
1.5 mg per person per day is below the effect level for humans. It should also be noted that the infrequency of exposure to residues further decreases the potential for adverse effects of antibiotic drug residues on the intestinal microflora. Specifically, although the ADI is calculated as the amount of drug residue that can be safely consumed daily throughout a person’s lifetime, in reality human exposure of this magnitude to antimicrobial residues is unlikely.

Since there is no validated model system for testing all antimicrobial agents, the Food and Drug Administration is currently funding studies designed to validate in vitro and in vivo models on the effects of low levels of antimicrobial residues on the human intestinal microflora. These studies will evaluate the adequacy of the ADI calculated from MIC data and the appropriateness of 1.5 mg per person per day as a threshold above which more data should be required. Although this approach has been discussed at meetings of the Joint FAO/WHO Expert Committee on Food Additives, it has not yet been accepted as feasible because it does not take into consideration the differences in biological activity and potency of the various antimicrobial drugs. It warrants further consideration since published results of experiments with antimicrobial agents in the 1 mg/kg range indicate that there is no observed effect on the human intestinal microflora.

4. Conclusions and recommendations

Continuous exposure of humans to antimicrobial agents in foods may alter the human intestinal microflora, and the effects of such agents on the intestinal flora should therefore always be investigated. However, more research is needed to determine whether there is a significant health risk from the consumption of foods containing low levels of residues, especially since antimicrobial substances present in food of animal and plant origin are consumed daily, probably in larger quantities than residues from the use of antimicrobial drugs in animals, with no apparent adverse effects on the intestinal microflora.

A microbiological evaluation should not necessarily be limited to one simple test system or to the equations given on pp. 79 and 80 in making a decision on safety. Such investigations may be based on in vitro or in vivo model systems, as outlined in Table 3, on the Food and Drug Administration approach, and/or on other relevant toxicological data. The extent and nature of these data sets may differ, depending both on the class of drug and on the extent to which it has been tested and/or used previously in humans and in animals. Types of information useful in evaluating an antimicrobial agent include its stability and bioavailability in the gut, its spectrum of activity against
different species of the gastrointestinal microflora, its influence on the barrier to colonization in *in vitro* or *in vivo* test systems, and its potential to cause gastrointestinal disturbances in animals or humans. This information could be used as an alternative approach in making regulatory decisions since it is extremely difficult to establish microbiological end-points in *in vitro* or *in vivo* test systems and to relate them to human food safety.

This approach would not necessarily exclude the use of the two equations proposed, but would provide a mechanism whereby additional data could be used to evaluate a drug, thereby permitting both the sponsor and the Committee some latitude in taking account of all relevant data available for the drug at the time of review. Such an approach would provide a more balanced risk assessment. Nevertheless, model systems used to assess the effects of low-level exposure to antimicrobial agents on the intestinal microflora, whether *in vivo* or *in vitro*, should be validated and designed for relevance to human exposure. Special attention should be given to the relevance of the calculations that have been performed based on MIC₉₀ values to the *in vivo* conditions in the human colon. If *in vitro* MIC₉₀ data continue to be generated and used for this purpose, the modified equation given on p. 79 should be used for calculating the microbiological ADI.

The Committee encourages the development of better *in vitro* and *in vivo* methods that are relevant for determining the effects of low concentrations of antimicrobial agents on the intestinal microflora in the human colon. While the Committee has used the formulae given on pp. 77 and 79 for establishing ADIs based on *in vitro* data, it recognizes that other *in vitro* or *in vivo* methods already exist or are under development and solicits comments that may improve the assessment of the potential risk of the dietary intake of residues from the use of antimicrobial drugs in animals.

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