EVALUATION OF CERTAIN VETERINARY DRUG RESIDUES IN FOOD

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

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Joint FAO/WHO Expert Committee on Food Additives
Geneva, 18–27 February 1997

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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:


**INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY**

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

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

A meeting of the Joint FAO/WHO Expert Committee on Food Additives was held at WHO Headquarters, Geneva, from 18 to 27 February 1997. The meeting was opened by Dr W. Kreisel, Executive Director, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization.

Dr Kreisel noted the increasing importance that the evaluations of the Committee play in the work of the Codex Alimentarius Commission and the World Trade Organization. Accordingly, the Committee has an increased responsibility to ensure that its evaluations are scientifically sound and are explained clearly. A great deal of progress has been made in recent years to increase the comprehensiveness of the reports and monographs and to explain the evaluations clearly; however, more clarity is always requested. The sponsoring Organizations are also responsible for publishing the reports and evaluations in a timely fashion.

The nine previous meetings of the Committee to consider veterinary drug residues in food (Annex 1, references 80, 85, 91, 97, 104, 110, 113, 119 and 125) had been held 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-seventh meeting of the Committee that meetings on this subject should be held annually (Annex 1, reference 125). 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 1955 (FAO Nutrition Meeting Report Series, No. 11, 1956; WHO Technical Report Series, No. 107, 1956), there have been 47 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 Tenth Session of the Codex Committee on Residues of Veterinary Drugs in Foods (2).

2. General considerations

2.1 Modification of the agenda

Imidocarb and porcine somatotropin were removed from the agenda because information for their review was not provided. This was the second time that the evaluation of these substances had been postponed.

The evaluation of residues of enrofloxacin was postponed due to the late submission of data.

2.2 Initiatives to promote transparency of the MRL-setting process of the Committee

The Committee considered several initiatives to make its process for setting MRLs more transparent and consistent. The Committee decided to review its entire evaluation policy, developed and documented in its reports, since its first meeting on residues of veterinary drugs in food in 1987 (Annex 1, reference 80). The ultimate aim of the Committee is to establish clearer principles for the allocation of MRLs for residues of veterinary drugs in food.

There are several areas in which further developments are highly desirable. Since the beginning of its work, for example, the Committee has allocated very few MRLs for therapeutic substances intended for so-called "minor" animal species. This problem has been identified previously by the Committee and competent authorities and other interested parties have expressed their concerns about it.

During its thirty-eighth meeting (Annex 1, reference 97), the Committee decided that, for edible tissues, at least two target tissues should be identified for which MRLs would be allocated. Nevertheless, competent authorities in charge of the control of veterinary drug residues in food may have problems in understanding the reasons for which all tissues are not allocated MRLs.

Recent meetings of the Committee have considered substances that have approved uses both as veterinary drugs and as pesticides for crop protection. In some situations where the compound has been previously reviewed by the Joint FAO/WHO Meeting on Pesticide
Residues, the Committee has had considerable difficulty in recommending MRLs for use of the substance as a veterinary drug in food-producing animals.

Some of the compounds used as veterinary drugs are highly lipophilic. As a consequence the highest levels of their residues are found in fat tissues. At its present meeting, the Committee discussed whether for these drugs MRLs for muscle and milk could be based on residue levels in fat.

Additional issues to be considered and further developed include guidelines for the performance of analytical methods, the relationship between the limit of quantification of analytical methods and MRLs, and the statistical determination of MRLs.

These issues will be discussed further at the fiftieth meeting of the Committee.

2.3 Standards for the generation of data

Most of the newer, comprehensive studies evaluated by the Committee have been performed according to appropriate standards for study protocol and conduct. Such standards are usually assured by adherence to codes of good laboratory practice (GLP) that have been specified by national governments or the Organisation for Economic Co-operation and Development (OECD). GLP codes require full documentation of the conduct and results of studies, including the results of chemical, toxicological and residue tests to ensure their validity.

At its present meeting, the Committee decided that it would specify whether studies had been performed according to present-day standards as exemplified by adherence to GLP codes. The guidelines or protocols used are cited in the monographs published in the *WHO Food Additives Series* and the *FAO Food and Nutrition Paper* series.

GLP codes were not always used in the design and conduct of older studies; however, many studies followed protocols that were adequate for assessing the safety of the substances being tested. When GLP codes are not cited, the Committee decided that it would comment, when appropriate, on the adequacy of the protocols for safety assessment and indicate in the toxicological monograph whether the study was inspected by a quality assurance unit, if such information is provided.

It should be emphasized, however, that, while standards for study protocol and conduct are valuable for ensuring the proper conduct of studies, they do not necessarily ensure scientific quality or the relevance of the design to resolving the scientific issue being addressed.
An inappropriate study may have been conducted according to GLP standards, while a study that does not meet GLP criteria may still be scientifically sound.

2.4 **Deadlines for the submission of data**

Not all data to be considered at the meeting were submitted in a timely fashion. Although the Secretariat specifies deadlines for the submission of data when announcing forthcoming meetings, such deadlines have been increasingly ignored in recent years. This makes it difficult to produce adequately reviewed monographs in time for the meeting.

The late submission of data has further undesirable consequences. If the Committee is forced to conclude that an adequate evaluation is impossible, the time dedicated by an expert to a preliminary review of the compound is wasted, and consideration of other drugs of equal priority on which data could have been supplied is delayed.

In consideration of the importance of complete, accurate and transparent evaluations of the Committee for the deliberations of the Codex Alimentarius and the implementation of the Sanitary and Phytosanitary and Technical Barriers to Trade Agreements of the World Trade Organization, the Committee stressed that data submitted after the specified deadline would normally be considered at a later meeting.

2.5 **Initiatives of FAO/WHO Member States and nongovernmental organizations**

FAO and WHO Member States or nongovernmental organizations wishing to submit information on an issue of general technical or scientific interest for consideration by the Committee should provide the Secretariat with a dossier on the issue not later than 2 months before the meeting.

When drafting the provisional agenda, the Secretariat will decide whether the issue will be considered, taking into account the workload of the meeting, and will inform the originator accordingly.

2.6 **Residues at the injection site**

The Committee reviewed a recent report prepared by S.C. Hathaway and the European Federation of Animal Health on residues of veterinary drugs at injection sites (3). The report made several recommendations to governmental agencies and standard-setting organizations
such as the Codex Alimentarius Commission. Several of these recommendations are applicable to the Committee; in summary, these are as follows:

1. A standard-setting process should be established for residues of veterinary drugs that reflects the public health significance of residues at the injection site.

2. International and government agencies should be encouraged to harmonize all regulatory aspects of standard-setting for residues of veterinary drugs that are related to injection sites.

3. It should be recognized that the likelihood of human exposure to residue levels that exceed the MRL at injection sites is extremely small.

4. The standard-setting process for veterinary drugs administered by the intramuscular route should be based on the safety evaluation for the possibility of acute adverse effects arising from ingestion of injection-site tissue from animals treated with a single dose of a given drug.

5. Injection-site tissue should be excluded from normal muscle when MRLs are established, when the particular residues have not been shown to cause adverse health effects in animals treated with a single dose of a given drug.

The Committee took note of these recommendations. At its thirty-eighth and forty-second meetings (Annex 1, references 97 and 110), the Committee stated the need for pharmacokinetic data on persistent residues at the injection site resulting from the use of long-acting formulations. At its forty-fifth meeting (Annex 1, reference 119), the Committee considered it desirable to standardize sampling procedures at the injection site in order to minimize the variability of the information obtained for review at future meetings and recommended the adoption of a specific sampling procedure. In the future, the Committee will note issues related to injection sites or to other sites to which a drug is applied locally, for consideration by the Codex Committee for Residues of Veterinary Drugs in Foods and national regulatory authorities.

3. Comments on residues of specific veterinary drugs

The Committee considered for the first time one antimicrobial agent and two insecticides. It reconsidered two anthelminthic agents, seven antimicrobial agents and one glucocorticosteroid. The
recommendations made with regard to these compounds and details of further information requested are summarized in Annex 2.

3.1 Anthelminthic agents

3.1.1 Moxidectin

Moxidectin was evaluated previously at the forty-fifth and forty-seventh meetings of the Committee (Annex 1, references 119 and 125).

Toxicological data
An ADI of 0–2μg per kg of body weight was established at the forty-fifth meeting of the Committee (Annex 1, reference 119). Moxidectin was not re-evaluated toxicologically at the present meeting.

Residue data
At the forty-fifth meeting (Annex 1, reference 119), MRLs were recommended for muscle, liver, kidney and fat in cattle, sheep and deer. The MRLs for deer were temporary. The sponsors provided new data on a large study in sheep for consideration at the forty-seventh meeting (Annex 1, reference 125). These data indicated that the residues in sheep muscle could exceed the MRL if the recommended dosing schedule for treatment of sarcoptic mange was used. The observation that the residue levels in ovine muscle were higher than those in bovine and deer muscle was likely to be due to the higher fat content in sheep muscle and the lipophilic nature of moxidectin. On the basis of this large study in sheep, an MRL of 50μg/kg in muscle was recommended at the forty-seventh meeting. At its Tenth Session, the Codex Committee on Residues of Veterinary Drugs in Food (2) requested the Expert Committee to consider whether the MRLs for cattle muscle could be harmonized with the higher MRL for sheep muscle, and also to consider new residue studies in cattle treated with multiple doses of moxidectin administered subcutaneously or as a pour-on formulation according to the recommended schedule.

Two studies in cattle treated with multiple doses of moxidectin were submitted by the sponsor. In the first study, 45 Angus steers were divided into one control group (nine animals) and six treatment groups (six animals per group). Four of the treatment groups received subcutaneous injections of moxidectin of 0.2mg per kg of body weight on days 0, 28, 56 and 84. Withdrawal periods for these four groups were 14, 21, 28 and 35 days following the last treatment, respectively. The two other groups received single subcutaneous injections of 0.2mg per kg of body weight on day 84. Withdrawal periods for these two groups were 14 and 35 days, respectively. Loin muscle and back
fat samples were collected for residue analysis. The upper 99% confidence limits for the concentration of moxidectin in fat of animals in the four multiple-treatment groups declined from 574µg/kg at 14 days to 97µg/kg at 35 days. Residue levels were below the limit of quantification (10µg/kg) in all muscle samples, except in one sample (13µg/kg) collected 14 days after the last treatment.

In the second study, 58 Hereford cattle were treated with a 0.5% pour-on formulation of moxidectin. Two control groups (three animals per group) were used. Six treatment groups (five animals per group) received the 0.5% solution at 0.1ml per kg of body weight (equivalent to 0.5mg per kg of body weight). The animals were dosed on days 0, 21, 42, 63 and 84, respectively. The six groups were killed 1, 7, 14, 21, 28 and 35 days after withdrawal of the drug, respectively. A further six treatment groups (four with three animals per group and two with five animals per group) received the 0.5% solution at twice the recommended dose (1.0mg per kg of body weight) with the same treatment and withdrawal periods. Samples of back fat, perirenal fat and liver were collected for residue analysis. The upper 99% confidence limits for the concentration of moxidectin residues in fat of animals that received the recommended treatment (0.5mg per kg of body weight) reached a maximum of 273µg/kg on day 7 and declined to 122µg/kg on day 35. Residues in liver tissue followed a similar pattern, reaching a maximum concentration of 35µg/kg on day 7 and declining to 5µg/kg on day 35.

**Maximum Residue Limits**

In the two studies described above, residue levels were higher in cattle treated with multiple doses of the subcutaneous preparation of moxidectin than in those treated with the pour-on preparation. The residue levels were highest in fat, followed by liver and muscle, respectively. If the recommended MRL for cattle fat of 500µg/kg is compared with data from the subcutaneous treatment study, residues would be expected to deplete to the MRL within approximately 17 days, based on the upper 99% confidence limits for the mean concentration of residues. The residue levels in liver and kidney tissue were not determined in the subcutaneous treatment study. However, on the basis of studies reviewed at the forty-fifth meeting (Annex 1, reference 119), in which cattle received a single subcutaneous injection of moxidectin, mean residue concentrations in liver and kidney would not be expected to exceed the respective MRLs of 100µg/kg and 50µg/kg when the residues in fat are at or below the MRL of 500µg/kg. Raising the MRL for cattle muscle to 50µg/kg is not
Table 1

<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>20</td>
<td>50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15</td>
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<tr>
<td>Liver</td>
<td>100</td>
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<td>Kidney</td>
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</tr>
<tr>
<td>Fat</td>
<td>500</td>
<td>667&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>79</td>
</tr>
</tbody>
</table>

<sup>a</sup> Expressed as parent drug.
<sup>b</sup> Based on a daily intake of 0.5 kg of meat made up of 0.3 kg of muscle, 0.1 kg of liver, 0.05 kg of kidney and 0.05 kg of fat.
<sup>c</sup> The marker residue accounted for 40% of the total residues in muscle, liver and kidney.
<sup>d</sup> The marker residue accounted for 75% of the total residues in fat.

warranted on the basis of the residue data available from the study in 36 cattle treated with multiple subcutaneous doses of moxidectin, which indicated that only one animal had residues in muscle above the limit of quantification of the analytical method at day 14. Accordingly, the Committee decided to retain the recommended MRL for cattle muscle of 20 µg/kg.

The Committee also decided to retain the MRLs recommended for liver, kidney and fat of cattle at its forty-fifth meeting. The parent drug is the marker residue and was considered to account for 40% of the total residues in the muscle, liver and kidney of cattle and 75% in fat, on the basis of the studies reported at the forty-fifth meeting, in which cattle were treated with a subcutaneous preparation or a pour-on formulation of radiolabelled moxidectin. From these values, the theoretical maximum daily intake of moxidectin residues is 79 µg (Table 1). This is considerably lower than the maximum ADI of 120 µg of moxidectin for a 60-kg person.

3.1.2 *Tiabendazole*<sup>1</sup> (*also known as thiabendazole*)

Tiabendazole is a benzimidazole used both as a broad-spectrum anthelmintic in animal species and for the control of parasitic infestations in humans. It was previously evaluated at the fortieth meeting of the Committee (Annex 1, reference 104), when an ADI of 0–100 µg per kg of body weight was established, based on a no-observed-effect level (NOEL) of 10 mg per kg of body weight per day for reduced body-weight gain in a 2-year dietary study in rats and decreased fetal

<sup>1</sup> *Tiabendazole* is the International Nonproprietary Name (INN) recommended by WHO. *Thiabendazole* is an alternative name that is used by certain national pharmacopoeias.
weight in a developmental toxicity study in rats and a safety factor of 100. At that meeting, the Committee asked to see the results from recently completed and ongoing toxicological studies in order to update the database on tiabendazole.

**Toxicological data**

Information from a number of new studies was available for assessment, including data on short-term and long-term toxicity, genotoxicity and reproductive toxicity. All of the studies were carried out according to appropriate standards for study protocol and conduct.

In a 12-month study, dogs were given no drug or tiabendazole at oral doses of 10, 40 or 160mg per kg of body weight per day. Anaemia, increased erythropoiesis and hypertrophy of thyroid follicular cells were noted at the highest dose. At 40 and 160mg per kg of body weight per day, vacuolation of epithelial cells of the bile duct and of renal tubular cells, cytoplasmic inclusions in the urinary bladder epithelium and increased concentrations of haemosiderin and erythropoiesis in the spleen were observed. The incidences of both cytoplasmic vacuolation of the gall-bladder epithelium and inspissated (thickened) bile in the villi of the gall bladder were increased in all treated groups. Similar observations have been reported previously both in dogs treated with tiabendazole and in untreated animals, including concurrent controls, which cast doubt on the toxicological significance of these effects. The NOEL was 10mg per kg of body weight per day, based on changes indicative of haemolytic anaemia.

Rats were given no drug or tiabendazole in the diet for 2 years at doses of 10, 30 or 90 mg per kg of body weight per day. The incidences of centrilobular hypertrophy in the liver and of hyperplasia of renal pelvic epithelial cells were increased in males at doses of 30 and 90 mg per kg of body weight per day and in females at a dose of 90 mg per kg of body weight per day. In the thyroid, hypertrophy and hyperplasia of follicular cells were observed at a dose of 90 mg per kg of body weight per day and the incidences of adenomas of thyroid follicular cells were increased in males at doses of both 30 and 90 mg per kg of body weight per day and in females at a dose of 90mg per kg of body weight per day. The NOEL was 10 mg per kg of body weight per day, based on the pathological changes in the liver, thyroid and kidney.

In a study of thyroid function, rats were given no drug or tiabendazole in the diet for 3 months at doses of 10, 90 or 270mg per kg of body weight per day. Treatment at a dose of 90 or 270mg per kg of body weight per day was associated with an increased rate of clearance of thyroxine, decreased levels of tri-iodothyronine and a concomitant
increase in the level of thyroid-stimulating hormone in serum. The Committee concluded that these findings are consistent with an indirect mechanism for stimulation of the thyroid gland by tiabendazole, leading to proliferative changes and subsequently to the formation of thyroid tumours in rats.

Tiabendazole induced numerical chromosomal aberrations in a variety of assays in vitro. This effect is thought to be due to inhibition of tubulin polymerization, which is a characteristic effect of many benzimidazoles. Tests for mutations in bacteria and for chromosomal aberrations in mouse bone marrow gave negative results.

In a 2-generation study of reproductive toxicity, rats were given no drug or doses of 10, 30 or 90 mg per kg of body weight per day in the diet. Reduced food intake and body-weight gain were observed in adult animals given doses of 30 and 90 mg per kg of body weight per day and in offspring at 90 mg per kg of body weight per day. Reproductive performance was not affected at any dose. The NOEL was 10 mg per kg of body weight per day, based on reduced maternal body-weight gain.

In a study of fetotoxicity, pregnant mice received no drug or tiabendazole at oral doses of 25, 100 or 200 mg per kg of body weight per day. No signs of fetotoxicity were observed. The numbers of live fetuses at doses of 100 and 200 mg per kg of body weight per day were decreased due to reduced implantation and the food intake and body-weight gain of the dams were also decreased at these doses. The NOEL was 25 mg per kg of body weight per day, based on reduced implantation. In a study in mice evaluated at the fortieth meeting (Annex 1, reference 104), fetal malformations were observed at doses of 240 mg per kg of body weight per day and above.

The results of these supplementary studies allowed the Committee to confirm the earlier evaluation. The NOELs in the 12-month study in dogs, the 2-year toxicity study in rats and the 2-generation study of reproductive toxicity in rats were all 10 mg per kg of body weight per day, identical to the NOEL identified previously that served as the basis for the ADI. The Committee applied a safety factor of 100 and confirmed the ADI of 0–100 μg per kg of body weight established at the fortieth meeting.

An addendum to the toxicological monograph was prepared.

**Residue data**

Residue data were not evaluated at the present meeting. Therefore, the Committee maintained the MRLs recommended at the fortieth meeting (Annex 1, reference 104) of 100 μg/kg for the edible tissues
(muscle, liver, kidney and fat) of cattle, pigs, goats and sheep, and 100μg/l for milk of cattle and goats. The total residues of tiabendazole are expressed as the sum of tiabendazole and 5-hydroxytiabendazole.

3.2 **Antimicrobial agents**

The Committee examined the results of *in vitro* studies of antimicrobial activity using relevant human gut microflora to calculate ADIs for some of the antimicrobial agents that were reviewed. The equation used for deriving these ADIs is based on that described in Annex 5 of the report of the thirty-eighth meeting of the Committee (Annex 1, reference 97) and modified at the forty-seventh meeting (Annex 1, reference 125).

The equation is as follows:

\[
\text{Upper limit of ADI (μg per kg of body weight)} = \frac{\text{MIC}_{50} \, (μg/g)^1 \times \text{Mass of colonic contents (g)}}{\text{Fraction of oral dose} \times \text{Safety factor} \times \text{Weight of human (kg) bioavailable}}
\]

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

A value of 220g is used for the mass of the colonic contents and a value of 60kg is used for the weight of an adult. The safety factor used to take account of uncertainty about the amount and relevance of data available for review may range from 1 to 10. A value of 1 is used when extensive relevant microbiological data are provided.

3.2.1 **Ceftiofur**

Ceftiofur was previously evaluated at the forty-fifth meeting of the Committee (Annex 1, reference 119), when an ADI of 0–50μg per kg of body weight was established, based on a microbiological end-point. At that meeting, the Committee recommended MRLs for ceftiofur of 200μg/kg for muscle, 2000μg/kg for liver, 4000μg/kg for kidney and

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1 Although \( \text{MIC}_{50} \) 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 \( \text{MIC}_{50} \) value is converted to these units, it is assumed that the density of the experimental medium is 1 g/ml.
600μg/kg for fat in both cattle and pigs, as well as 100μg/l for cows’ milk, expressed as desfuroylceftiofur. These MRLs accounted for a maximum daily intake of 640μg, while the ADI set by the Committee resulted in a maximum ADI of 3000μg for a 60-kg adult.

Residue data
At its forty-fifth meeting, the Committee agreed to consider additional information on the depletion of ceftiofur if such information should become available in the future, particularly with respect to the depletion of the marker residue in treated animals 12–72 hours after withdrawal. At its present meeting, the Committee received the following new studies for review, all conducted according to appropriate standards for study protocol and conduct.

Cattle. Six cattle each received five successive daily intramuscular injections of 2.45mg per kg of body weight of [14C]ceftiofur free acid equivalents. The cattle were slaughtered 12 hours after the final injection. The concentrations of the total residues (based on total radioactivity recovered) were as follows: 0.15–0.29mg/kg in muscle, 1.2–1.8mg/kg in liver, 6.0–8.3mg/kg in kidney and 0.35–0.46mg/kg in fat. Residues in the injection sites ranged from 0.6–2.7mg/kg in the first injection site to 13–48mg/kg in the fifth injection site.

The marker residue accounted for approximately half of the total residue present in all edible tissues at slaughter 12 hours after the final dose.

Twelve cattle each received five successive daily subcutaneous injections of 2.2mg per kg of body weight of ceftiofur hydrochloride. The cattle were slaughtered 12 hours after the final treatment. Residues in edible tissues, determined as desfuroylceftiofur, were 0.12–0.29mg/kg in muscle, 3.7–5.6mg/kg in kidney and 0.77–1.8mg/kg in liver. Residues in fat were not measured. The results were consistent with those observed in studies where the drug was administered by intramuscular injection.

Sixty male calves were used in a trial conducted at two separate locations. At each location, 15 cattle received five successive daily intramuscular injections of 2.0mg per kg of body weight of ceftiofur as ceftiofur sodium. The remaining 15 cattle received 4.0mg per kg of body weight of ceftiofur as ceftiofur sodium. Samples of the edible tissues and injection-site tissue were collected from each of three animals slaughtered at 1, 3, 15, 20 or 25 days after the final injection and analysed for desfuroylceftiofur by high-performance liquid chromatography (HPLC). No desfuroylceftiofur residues were found in muscle of cattle treated at 2mg per kg of body weight at any sampling
date. In kidney, the levels of residues ranged from 0.27 to 0.70 mg/kg at day 1, declining to <0.05–0.17 mg/kg at day 3 and to undetectable levels in subsequent samples. Residues in liver were more persistent, declining from a level of 0.10–1.40 mg/kg at day 1 to <0.05–0.46 mg/kg at day 3 and to <0.05 mg/kg by day 15. Levels of residues in fat were 0.07–0.15 mg/kg at day 1, <0.05–0.10 mg/kg at day 3 and <0.05 mg/kg at day 15. In animals dosed at 4 mg per kg of body weight, residue levels in muscle were <0.05–0.08 mg/kg at day 1 and <0.05 mg/kg on subsequent sampling dates. Levels of residues in kidney, liver and fat were generally higher than those in animals dosed at 2.0 mg per kg of body weight, but had depleted in all tissues by day 15, except for liver (<0.05–0.16 mg/kg). Levels of residues in the injection-site tissue were 4.2–90 mg/kg at day 1 in animals dosed at 4.0 mg per kg of body weight, but had declined to <0.05–0.38 mg/kg at 3 days after the final injection. In animals dosed at 2.0 mg per kg of body weight, residues in the injection-site tissue were 0.36–9.1 mg/kg at day 1, but had declined to 0.05–0.32 mg/kg at 3 days after the final treatment.

Pigs. In the first phase of a study using [14C]ceftiofur monohydrochloride, six pigs each received three successive daily intramuscular injections of 5.0 mg per kg of body weight, while another six pigs received three successive daily intramuscular injections of 7.5 mg per kg of body weight. Animals were slaughtered 12 hours after administration of the last injection and tissues were collected and analysed for both total radioactivity and desfuroylceftiofur. In the second phase of the study, 12 pigs each received three successive daily intramuscular injections of 3.0 mg per kg of body weight of ceftiofur and were slaughtered in groups (four per group) 12, 72 and 120 hours after the last injection. Levels of residues increased with increasing dosage, but the marker residue was found only in the samples collected from animals slaughtered 12 hours after the final injection, with the exception of kidney from one pig in the group killed at 72 hours, which contained detectable residues. In the group dosed at 3.0 mg per kg of body weight, the levels of desfuroylceftiofur residues were 0.20–0.35 mg/kg in muscle, 0.55–0.74 mg/kg in liver, <0.05–1.76 mg/kg in kidney and 0.43–0.57 mg/kg in fat at 12 hours after the final injection, while the corresponding values for the group dosed at 5.0 mg per kg of body weight were 0.40–0.72 mg/kg in muscle, 0.68–1.18 mg/kg in liver, 2.54–3.64 mg/kg in kidney and 0.29–1.29 mg/kg in fat, and those for the group dosed at 7.5 mg per kg of body weight were 0.55–0.87 mg/kg in muscle, 1.04–1.59 mg/kg in liver, 3.24–4.98 mg/kg in kidney and 0.62–1.88 mg/kg in fat. Desfuroylceftiofur accounted for 67% of the total residues (determined as [14C]ceftiofur) in muscle; the corresponding values for liver, kidney and fat were 50%, 33% and 40%, respectively.
Studies were conducted at two different geographical locations in 60 castrated pigs, which received three successive daily intramuscular injections of 3.0 or 6.0 mg per kg of body weight of ceftiofur (15 animals per treatment group). Three animals from each group were slaughtered at 1, 3, 7, 10 or 15 days after the third injection and samples of muscle, liver, kidney, fat and injection-site tissue (muscle) were collected and analysed by HPLC for the marker residue, desfuroylceftiofur. In the groups dosed at 3.0 mg per kg of body weight per day, residue levels were 0.12–0.22 mg/kg in muscle, 0.12–0.20 mg/kg in liver, 0.34–0.68 mg/kg in kidney, 0.20–0.32 mg/kg in fat and 0.09–0.42 mg/kg in injection-site tissue at day 1 after the final injection. In the groups dosed at 6.0 mg per kg of body weight per day, residue levels were 0.17–0.28 mg/kg in muscle, 0.18–0.31 mg/kg in liver, 0.48–0.75 mg/kg in kidney, 0.26–0.37 mg/kg in fat and 0.43–0.78 mg/kg in injection-site tissue at day 1. In animals dosed at 3.0 mg per kg of body weight, only kidney (0.07 mg/kg) and fat (0.08 mg/kg) from one pig contained detectable residues at day 3. In contrast, residues were detectable in all tissues except muscle from animals dosed at 6.0 mg per kg of body weight. The distribution was as follows: <0.05–0.06 mg/kg in liver, 0.06–0.10 mg/kg in kidney and <0.05–0.10 mg/kg in fat. There were no detectable residues in any tissues at either dose level at 7 days after the final injection. As in the studies evaluated at the forty-fifth meeting, the residues were highest and most persistent in kidney.

**Maximum Residue Limits**

In reaching its decision on the MRLs for ceftiofur, the Committee took into account the following:

- The ADI of 0–50 μg per kg of body weight established by the Committee at its forty-fifth meeting, based on a microbiological end-point.
- A significant proportion of the total residue is considered to be inactive because the β-lactam ring is no longer intact. MRLs are expressed as the marker residue, desfuroylceftiofur, which accounts for all active residues.
- Residues measured as desfuroylceftiofur are near or below the limit of detection (0.05 mg/kg) in muscle and fat and <0.5 mg/kg in liver and kidney at 3 days after treatment in both cattle and pigs in the studies provided.
- On the basis of the residue-depletion studies submitted at the present meeting, the MRLs established for muscle and kidney by the Committee at its forty-fifth meeting may be exceeded in some treated pigs and cattle at 12 hours after the final injection, even if
treatment is in accordance with recommended good practice in the use of veterinary drugs.

- The MRLs for pigs and cattle were harmonized, on the basis of the residue-depletion studies submitted.
- The drug is used for therapeutic purposes only.
- No new data were submitted on residue levels in milk.

On the basis of the above considerations, the Committee recommended MRLs for ceftiofur of 1000µg/kg for muscle, 6000µg/kg for kidney and 2000µg/kg for fat in both cattle and pigs, expressed as desfuroyloceftiofur. In addition, the Committee reaffirmed the MRLs recommended for ceftiofur at its forty-fifth meeting of 2000µg/kg for liver in both cattle and pigs and 100µg/l for cows’ milk.

The MRLs recommended above would result in a theoretical maximum daily intake of 1050µg of microbiologically active ceftiofur residues, based on a daily food intake of 300g of muscle, 100g of liver, 50g each of kidney and fat, and 1.5l of milk (Annex 1, reference 85).

3.2.2 Danofloxacin

Danofloxacin is a fluoroquinolone antimicrobial agent with wide-spectrum antibacterial activity. It is administered to poultry, cattle and pigs for the treatment of respiratory infections. Danofloxacin had not been previously evaluated by the Committee.

Toxicological data

The Committee considered data from a range of studies on danofloxacin, including the results of studies on its pharmacokinetics, acute, short-term and long-term toxicity, carcinogenicity, reproductive toxicity, genotoxicity and antimicrobial activity. The results of studies on the acute and short-term toxicity, genotoxicity, reproductive toxicity and antimicrobial activity of the metabolite desmethyldanofloxacin were also available to the Committee. The mesilate salt of danofloxacin and desmethyldanofloxacin was used in these studies. Most of the studies critical for the evaluation were carried out in accordance with appropriate standards for study protocol and conduct.

Danofloxacin was rapidly absorbed after oral administration to chickens and pigs and after intramuscular administration to pigs and cattle. Only one study was carried out in which the bioavailability of danofloxacin was determined; in this study, the bioavailability after oral administration of 5mg per kg of body weight to pigs was approximately 90%. Urine and faeces contained approximately equal amounts of danofloxacin and its metabolites.
In cattle, dogs and rats, unchanged danofloxacin was the main substance present in the faeces; smaller amounts of desmethyldanofloxacin were found. Danofloxacin was also the main component in urine. Desmethyldanofloxacin, danofloxacin-N-oxide and the β-glucuronide of danofloxacin were also found in urine. A piperazine-ring degradation product was present in the bile of cattle but was found in only trace amounts in the bile of rats and dogs. Residues of both danofloxacin and desmethyldanofloxacin were found in liver samples from rats, dogs, chickens, cattle and pigs. The Committee considered that the metabolism of danofloxacin was very similar in laboratory animals and in the three target species.

Single oral doses of both danofloxacin and desmethyldanofloxacin were slightly toxic to rats and mice, with oral LD₅₀ values in the range 1500 to 2000 mg per kg of body weight. Signs of toxicity typical of stimulation of the central nervous system were observed prior to death.

Several exploratory studies were carried out in rats given danofloxacin in the feed and by gavage, and in rabbits given danofloxacin by gavage. In most of these studies caecal dilatation was noted at doses of 25 mg per kg of body weight per day and above. In rats, increased incidences of crystals in the urine were observed at doses of 75 mg per kg of body weight per day and above. There were no corresponding pathological changes in the kidneys. In a 3-month study in which danofloxacin was administered to rats in the diet, degenerated germinal cells were found in the epididymides of all 15 males given 300 mg per kg of body weight per day and in 9 of 15 males given 150 mg per kg of body weight per day. In view of the high doses used, the results of these studies were not considered useful for evaluating the safety of danofloxacin.

Rats were exposed to danofloxacin in utero and during lactation by oral administration to the dams of no drug or doses of 25, 75 or 150 mg per kg of body weight per day. The offspring received the same oral doses for an additional 3 months after weaning. In females, there was a dose-related increase in proteinuria which correlated with the finding of tubular nephropathy in individual animals. In males dosed at 75 and 150 mg per kg of body weight per day, both the absolute and relative testicular weights were 10% lower than those of untreated controls. In a 3-month follow-up study in which lower doses were used, no effects attributable to treatment were observed at the highest dose (6.25 mg per kg of body weight per day). The Committee concluded that the overall NOEL for renal tubular nephropathy in rats was 6.25 mg per kg of body weight per day.
Rats were exposed to desmethyldanofoxacin *in utero* and during lactation by oral administration to the dams of no drug or doses of 1, 2.5 or 6.25 mg per kg of body weight per day. The offspring received the same oral doses or no drug for an additional 3 months after weaning. No adverse effects were observed at any dose.

Six-month-old dogs were given oral doses of 5, 10 or 25 mg per kg of body weight per day of danofoxacin or no drug in gelatin capsules for 3 months. Those given doses of 10 and 25 mg per kg of body weight per day showed signs of joint pain. Pathological examination revealed arthropy characterized by areas of cartilage separation and erosion in all treated groups, and the severity of the lesions was dose-related. A second 3-month study was carried out in 5-month-old dogs given oral doses of 1 or 2.4 mg per kg of body weight per day of danofoxacin or no drug in gelatin capsules. There was no evidence of arthropy or any other treatment-related effect. On the basis of the results of the two studies, the Committee concluded that the NOEL for arthropy in dogs was 2.4 mg per kg of body weight per day.

A further 3-month study was carried out in which 4–6-month-old dogs were given oral doses of 2.5, 5 or 10 mg per kg of body weight per day of desmethyldanofoxacin or no drug in gelatin capsules. One of three males given the highest dose and one of three females given the lowest dose showed signs of pain on examination. In the male, morphological changes were found in the articular cartilage of one joint. When the study was repeated using doses of 0.25 or 0.5 mg per kg of body weight per day of desmethyldanofoxacin, one of three males given a dose of 0.5 mg per kg of body weight per day was found to have histopathological changes in the right knee that were typical of quinoline-induced arthropy. The NOEL for desmethyldanofoxacin was 0.25 mg per kg of body weight per day, based on arthropy.

In a 102-day range-finding study, mice were fed diets containing no drug or danofoxacin at concentrations equivalent to 150, 300 or 600 mg per kg of body weight per day. At the highest dose males showed a reduction in body-weight gain and haematological parameters were decreased in both sexes. Increased kidney weights were observed in females. Caecal dilatation was observed at all doses in both sexes, and two of 30 mice given the highest dose had inflammation of the caecum.

In a 2-year study of carcinogenicity, mice were fed diets containing no drug or doses of danofoxacin equivalent to 10, 50 or 100 mg per kg of body weight per day. Females given the highest dose gained more weight than the controls and showed increased absolute kidney
weights. There were no adverse effects on haematological parameters. No increase in tumour incidence was observed at any dose.

In a 2-year study of carcinogenicity, rats were fed diets containing no drug or doses of danofloxacin equivalent to 10, 50 or 100 mg per kg of body weight per day. At the highest dose, erythrocyte volume fraction and haemoglobin and lymphocyte levels were decreased in females; in males serum alanine aminotransferase activity was increased and serum globulin levels were reduced. Serum sorbitol dehydrogenase activity was increased in males given 50 or 100 mg per kg of body weight per day. The relative testicular weight was reduced and increased oligospermia and abnormal epididymidal content were observed in the highest-dose group. Caecal enlargement was noted at all doses, but was not correlated with any microscopic findings. An increased incidence of papillary oedema was noted in the kidneys of rats given doses of 100 mg per kg of body weight per day. There was a significant positive trend in the incidence of granular-cell tumours of the uterus and vagina in treated female rats. These tumours were distinguished from granular-cell foci by their larger size and the compression of adjacent tissue, but the morphology of these lesions was essentially similar and the Committee considered that it was appropriate to combine the incidences of these uterine and vaginal foci and tumours. There was no significant trend in the combined incidence across groups. There was a significant positive trend in the incidence of pituitary adenomas in treated females, but the number of rats with these lesions was within the range in historical controls. None of the trends in tumour incidence was significant when corrected for multiple comparisons. The Committee concluded that neither the granular-cell lesions of the uterus and vagina nor the pituitary adenomas were indicative of a carcinogenic response to treatment with danofloxacin.

The genotoxic properties of danofloxacin were investigated in vitro in assays for gene mutation in bacteria and in mammalian cells, unscheduled DNA synthesis and cytogenetic alterations in mammalian cells and in vivo, in assays for cytogenetic effects. All of the tests gave negative results, except for an assay of cytogenetic alterations in human lymphocytes in vitro. The clastogenicity observed in vitro was reduced or abolished by addition of magnesium sulfate to the culture medium and/or washing the cells after treatment to remove danofloxacin and appeared to be associated with the cation-chelating properties of danofloxacin. There was no evidence of clastogenicity in vivo. Desmethyldanofloxacin induced a significant increase in unscheduled DNA synthesis in two independent assays in primary rat hepatocytes in vitro; however, negative results were obtained with
desmethyldanofloxacin in an assay for unscheduled DNA synthesis and in a test for micronucleus formation in vivo. Thus, although desmethyldanofloxacin induced unscheduled DNA synthesis in vitro, this genotoxic potential did not appear to be expressed in vivo.

In a 2-generation study of reproductive toxicity, rats received doses of 25, 75 or 150mg per kg of body weight per day of danofloxacin or no drug by gavage. In the parental generation, maternal body-weight gain was reduced at the highest dose, and these dams had fewer implantation sites and produced fewer viable pups. These effects were observed at lower doses with subsequent matings. At the second mating of the first-generation animals, the pregnancy rate was adversely affected in all treated groups. No NOEL could be identified.

A 3-generation study of reproductive toxicity was carried out in which rats were given danofloxacin at doses of 1, 2.5, 6.25 or 150mg per kg of body weight per day or no drug by gavage. In animals given doses of 150mg per kg of body weight per day, the number of mated females and the pregnancy rate were reduced, the duration of gestation was increased, and the litter sizes and pup weights were reduced; this group was killed before the second mating of the first generation. In this study, in which lower doses were used than in the study described above, the NOEL for reproductive toxicity was 6.25mg per kg of body weight per day.

No adverse effects were observed in a 3-generation study of reproductive toxicity in rats in which doses of up to 6.25mg per kg of body weight per day of desmethyldanofloxacin or no drug were administered by gavage.

In a study of developmental toxicity in mice, there was no evidence of teratogenicity when danofloxacin was administered by gavage at doses of up to 200mg per kg of body weight per day on days 6–13 of gestation. The highest dose was toxic to the dams, reducing body-weight gain. It was also fetotoxic, producing a reduction in mean fetal weight and an increased incidence of delayed ossification. The study was compromised by the small numbers of gravid dams in all groups, including the controls. The NOEL for both maternal toxicity and fetotoxicity was 100mg per kg of body weight per day.

In a study of developmental toxicity in rats, oral doses of 50, 100 or 200mg per kg of body weight per day of danofloxacin or no drug were administered on days 6–15 of gestation. Maternal body-weight gain and food consumption were reduced at doses of 100 and 200mg per kg of body weight per day. These doses were also fetotoxic, producing an
increased incidence of delayed ossification and dilatation of the cerebral ventricles. The NOEL for both maternal toxicity and fetotoxicity was 50mg per kg of body weight per day.

In a study of developmental toxicity in rabbits, oral doses of 2.5, 7.5 or 15 mg per kg of body weight per day of danofloxacin or no drug were administered on days 6–20 of gestation. In animals dosed at 15 mg per kg of body weight per day, maternal body-weight loss, reduced food consumption and abortion were observed. There was no evidence of teratogenicity or fetotoxicity at any dose. The NOEL for maternal toxicity was 7.5 mg per kg of body weight per day.

**Microbiological data**

The minimum danofloxacin concentration resulting in 50% inhibition (MIC$_{50}$) was determined for 64 isolates of the six predominant genera of human intestinal anaerobic microflora (Bacteroides, Fusobacterium, Clostridium, Eubacterium, Bifidobacterium and Peptostreptococcus). In addition, data were provided for the facultative anaerobes (Lactobacillus spp., Proteus spp. and Escherichia coli). Although E. coli and Proteus spp. were the most sensitive organisms, the Committee agreed that they should not be taken into account in the calculation of the MIC$_{50}$ because they are not predominant species in the human intestine. Instead, the Committee derived the mean MIC$_{50}$ from data available on 32 strains of the most sensitive relevant genera isolated from the human gastrointestinal tract, in this case Eubacterium spp., Bifidobacterium spp. and Peptostreptococcus spp. The mean MIC$_{50}$ for these strains was 1 µg/ml. This figure was used in calculating an ADI based on antimicrobial activity from the formula described in section 3.2:

\[
\text{Upper limit of ADI} = \frac{1 \mu g/g^a \times 220 g^b}{0.1^c \times 1^d \times 60kg^e} = 37 \mu g \text{ per kg of body weight}
\]

The Committee noted that the metabolite desmethyldanofloxacin was between one-quarter and half as active as danofloxacin against

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a The mean MIC$_{50}$ for the sensitive relevant species in the human intestine, in this case Eubacterium spp., Bifidobacterium spp. and Peptostreptococcus spp.
b The mass of the colonic contents.
c The fraction of the oral dose available to the gut microflora was determined to be about 10%, based on a study in pigs given an oral dose of 5 mg per kg of body weight, in which approximately 90% was absorbed. The finding that danofloxacin is strongly bound to cattle faeces was considered by the Committee to add confidence to the use of this value.
d A safety factor of 1 was used because the Committee considered that sufficient relevant microbiological data had been provided.
e The weight of an adult person.
the same isolates. The Committee also noted that danofloxacin belongs to a group of fluoroquinolones that is active against aerobic Gram-negative bacteria and that the main components of the human gastrointestinal flora are largely unaffected by these compounds. Therefore, the Committee decided to base the ADI on the toxicity of danofloxacin and not on its effect on the intestinal flora. Moreover, the toxicological end-point would give the lowest ADI.

The Committee established an ADI of 0–20µg per kg of body weight based on the NOEL of 2.4mg per kg of body weight per day for arthropathy in a 3-month study in immature dogs and a safety factor of 100. The ADI was rounded to one significant figure, which is the standard practice (Annex 1, reference 91, section 2.7).

The Committee noted that the NOEL for arthropathy in studies with desmethyldanofloxacin was 0.25mg per kg of body weight per day. The pharmacokinetic and metabolic studies showed that dogs that received oral doses of danofloxacin were also exposed systemically to the metabolite desmethyldanofloxacin. The Committee therefore concluded that it was not necessary to calculate a separate ADI for desmethyldanofloxacin. It agreed, however, that the approximately 10-fold higher toxicity of the metabolite should be taken into account when recommending MRLs for danofloxacin, as consumers may consume desmethyldanofloxacin in liver.

A toxicological monograph was prepared.

Residue data
Danofloxacin was found to be metabolized in a quantitatively similar manner in rats, dogs, cattle, chickens and pigs via pathways that were predictable from studies reported for other quinolones. The parent drug, danofloxacin, accounted for the majority of the residues found in edible tissues following treatment of chickens, cattle and swine. The metabolite desmethyldanofloxacin was the main residue in liver of the three target species. No other metabolites were present in significant quantities in liver.

Residue-depletion studies using [3H]-labelled danofloxacin and unlabelled danofloxacin were carried out in cattle, pigs and chickens using the doses recommended for normal therapeutic treatment.

Cattle. [3H]Danofloxacin was administered to mature cattle once daily by intramuscular injection at a dose of 1.25mg per kg of body weight for 5 successive days. Total residues, unchanged danofloxacin and desmethyldanofloxacin were determined in edible tissues collected at 12, 24, 36, 48, 60 and 72 hours after the last treatment. The highest
concentrations of total residues, as measured by radioactivity, were found in the liver. At 12 hours after treatment the concentration was about 1000µg/kg and it decreased to about 200µg/kg at 72 hours with a half-life of approximately 26 hours. Total residues depleted more rapidly in the other tissues; the half-lives were 14 hours in kidney, 17 hours in muscle, and 11 hours at the injection site. Analysis of the liver for danofloxacin and desmethyldanofloxacin showed that whereas the parent drug accounted for about 21–32% of the total radioactivity, the proportion accounted for by the metabolite declined gradually from 41% at 12 hours to 14% at 72 hours. At 48 hours after dosing the total residue concentrations in the injection-site muscle were similar to those in other muscle samples.

Residue-depletion studies using unlabelled danofloxacin were carried out in five groups of cattle (six animals per group) which received five successive daily intramuscular injections of 1.25mg per kg of body weight of danofloxacin. Samples of muscle, liver, kidney, fat and injection-site tissue were obtained at 12, 36, 60, 84 and 120 hours after the last injection and were analysed by HPLC for the presence of danofloxacin and desmethyldanofloxacin. The residues depleted at rates comparable to those of the total residues. At 5 days after withdrawal, danofloxacin was measurable only in the liver (13µg/kg) and the level of the metabolite was below the limit of quantification of the assay (10µg/kg) in all tissues.

Pigs. Residue-depletion studies using [³H]danofloxacin were carried out in five groups of pigs (four animals per group) which received five successive daily intramuscular injections of 1.25mg per kg of body weight of danofloxacin. Samples of muscle, liver, kidney, fat and injection-site tissue were obtained at 12, 24, 48 and 168 hours after the last injection and were analysed by HPLC for the presence of danofloxacin and desmethyldanofloxacin. The highest concentrations of radioactive substances were found in the liver (960–1021µg/kg) and kidney (824–991µg/kg) at 12 hours after withdrawal of the drug; these tissues continued to have the highest radioactive content for up to 168 hours after dosing. The radioactivity declined with a half-life of 22–24 hours in the muscle, fat and injection site, 41 hours in the kidney and 72 hours in the liver. The contents of danofloxacin and desmethyldanofloxacin in the pooled liver samples were measured by an HPLC assay. At 12 hours after dosing both compounds were present in equal amounts, but at later times (>48 hours) desmethyldanofloxacin accounted for the highest proportion (about 90%) of the total residues.

Residue-depletion studies using unlabelled danofloxacin were carried out in five groups of pigs (four animals per group) which received
three successive daily intramuscular injections of 1.25 mg per kg of body weight of danofloxacin. Samples of muscle, liver, kidney, fat and injection-site tissue were obtained at 2, 6, 10, 14 and 18 days after the last injection and were analysed by HPLC for the presence of danofloxacin and desmethyldanofloxacin. Residues of danofloxacin depleted rapidly in all tissues, so that by the first sampling period at 2 days after final dosing, danofloxacin was present only in very low concentrations (<40 µg/kg) and by 6 days after final dosing, danofloxacin was not detectable in any tissue. At 2 days after the last injection, desmethyldanofloxacin was present in the liver (408–1065 µg/kg) after which concentrations continued to decline, but were still detectable at 18 days after treatment (34–147 µg/kg).

*Chickens.* [³H]Danofloxacin was administered in drinking-water to 23 three-week-old chickens at a dose equivalent to 5.0 mg per kg of body weight per day for 5 days. Birds were killed at 6, 12, 24 and 48 hours after withdrawal of the treatment and the total residues in their edible tissues were measured. The highest concentrations of total residues were at 6 hours and were consistently found in both the liver (457–850 µg/kg) and the kidney (291–641 µg/kg). The half-lives were similar (9–11 hours) for the total residues in liver, kidney and muscle and slightly longer (18 hours) in skin/fat tissue. In pooled liver homogenates danofloxacin was the most abundant residue (47–61% of the total residue) at all withdrawal times and desmethyldanofloxacin was the major metabolite (13–20% of the total residue).

The depletion of unlabelled danofloxacin from chicken tissues was evaluated in a study in which danofloxacin mesilate soluble powder was administered in drinking-water to 30 three-week-old chickens at a dose of 5.0 mg per kg of body weight per day for 3 days. Muscle, liver and skin/fat samples were collected at 6, 12, 18, 24 and 36 hours after withdrawal of treatment and were analysed by HPLC for the presence of danofloxacin and desmethyldanofloxacin. Depletion of danofloxacin and desmethyldanofloxacin occurred rapidly in chicken tissue following drug withdrawal. Liver always contained the highest concentrations of both danofloxacin (157–319 µg/kg) and desmethyldanofloxacin (35–193 µg/kg) at 6 hours after withdrawal. All other tissues contained lower concentrations of danofloxacin than did the liver, and desmethyldanofloxacin was found only in the liver. The concentrations of danofloxacin declined in muscle, kidney and fat to <25 µg/kg at 36 hours after withdrawal. In muscle the maximum concentration of danofloxacin was 91 µg/kg at 6 hours in one bird but by 18 hours the residue level was undetectable (<25 µg/kg). At 6 hours after withdrawal, levels of residues of parent drug in skin/fat were
61–235\(\mu\)g/kg and had decreased to <25–41\(\mu\)g/kg by 36 hours after withdrawal. The concentrations of both danofloxacin and its metabolite in liver decreased rapidly; at 36 hours after withdrawal the concentration of the parent drug was 41\(\mu\)g/kg and that of the metabolite <10\(\mu\)g/kg.

**Analytical methods**

The Committee considered that the analytical method submitted by the sponsor was adequate for routine monitoring purposes. The samples were extracted by acid hydrolysis and solvent partition from tissue homogenates or fat, followed by HPLC with fluorescence detection. The method was suitable for measuring danofloxacin concentrations at the \(\mu\)g/kg level in cattle and poultry tissue. The assay had a limit of quantification of 10\(\mu\)g/kg and was linear between 10 and 500\(\mu\)g/kg in liver, muscle, kidney, fat and injection-site muscle. Recovery of danofloxacin from all fortified tissues was close to 100%. The accuracy of the method was between 99 and 103% and the precision, expressed as the coefficient of variation, was 2% or less. The method was specific for danofloxacin in the presence of several other fluoroquinolones, including desmethyldanofloxacin, enrofloxacin, ciprofloxacin, norfloxacin, ofloxacin, sarafloxacin, marbofloxacin and sparfloxacin.

A method to confirm the presence of danofloxacin in liver tissues used HPLC combined with mass spectrometry.

**Maximum Residue Limits**

In reaching its decision on MRLs for danofloxacin, the Committee took into account the following:

- An ADI of 0–20\(\mu\)g per kg of body weight was established, based on a toxicological end-point. This would result in a maximum ADI of 1200\(\mu\)g for a 60-kg person.
- The parent drug is the marker residue.
- The metabolite desmethyldanofloxacin is considered to be ten times as toxic as the parent drug.
- Only the toxicological activity of the parent drug and desmethyldanofloxacin are known. Therefore all the residues, including the bound residues, are assumed to be of toxicological concern. The unidentified residues are assumed to have the same toxicity as the parent drug.
- Liver and muscle were considered to be the appropriate target tissues.
- Desmethyldanofloxacin occurs only in the liver.
• The proportion of the total residues accounted for by danofloxacin and desmethyldanofloxacin varies between different species.

• The sum of all the residues covered by the ADI expressed as danofloxacin equivalents is given by the formula:

\[ \text{Total residues} = \text{residues of danofloxacin + unidentified residues expressed as danofloxacin equivalents} + \text{residues of desmethyldanofloxacin expressed as danofloxacin equivalents} \]

The Committee recommended MRLs of 200\(\mu\)g/kg for muscle and 400\(\mu\)g/kg for liver and kidney in both cattle and chickens, as well as 100\(\mu\)g/kg for fat in cattle and for fat/skin (in normal proportions) in chickens, expressed as danofloxacin.

The Committee recommended MRLs of 100\(\mu\)g/kg for muscle, 50 \(\mu\)g/kg for liver, 200\(\mu\)g/kg for kidney and 100\(\mu\)g/kg for fat in pigs, expressed as danofloxacin.

The total residues were calculated as danofloxacin using the above formula to give the theoretical maximum daily intake for the tissues for each species (Table 2).

### 3.2.3 Dihydrostreptomycin and streptomycin

Dihydrostreptomycin and streptomycin are aminoglycoside antibiotics used for the treatment of bacterial infections in food-producing animals. The two compounds were evaluated together at the twelfth and forty-third meetings of the Committee (Annex 1, references 17 and 113). At its forty-third meeting, the Committee established a temporary ADI of 0–30\(\mu\)g per kg of body weight for the combined residues of both dihydrostreptomycin and streptomycin, based on a NOEL of 5\(\text{mg}\) per kg of body weight per day in a 2-year oral toxicity study in rats and a safety factor of 200. At its forty-third meeting, the Committee concluded that additional information was needed to assess the potential for effects on fertility and perinatal and post-natal toxicity, which was requested for evaluation in 1997. It also requested the following information on residues for evaluation in 1997:

1. An evaluation report or results of experimental studies on the metabolism of dihydrostreptomycin and streptomycin.
2. Data on residues of streptomycin and dihydrostreptomycin in eggs.
3. Results of studies to determine the relationship between the antimicrobial activity of the residues and their concentration, as measured by specific chemical methods.
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Recommended MRL (µg/kg)</th>
<th>Estimate of total residues (µg/kg danofloxacin equivalents)</th>
<th>Theoretical maximum daily intake (µg danofloxacin equivalents)</th>
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<tr>
<td></td>
<td></td>
<td>Danofloxacin</td>
<td>Desmethyldanofloxacin</td>
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<tr>
<td><strong>Cattle</strong></td>
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<td>Muscle</td>
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<td><strong>Chickens</strong></td>
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<td>(in normal proportions)</td>
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<tr>
<td><strong>Total</strong></td>
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</table>

* Expressed as parent drug.
* Desmethyldanofloxacin is considered to be 10 times as toxic as the parent drug.
* The unidentified residues are assumed to have the same toxicity as the parent drug.
* Based on a daily intake of 0.5 kg of meat made up of 300 g of muscle, 100 g of liver, and 50 g each of kidney and fat.
* The marker residue and desmethyldanofloxacin each accounted for 25% of the total residues in bovine liver.
* The marker residue accounted for 50% of the total residues in bovine kidney.
* The marker residue accounted for 40% of the total residues in porcine muscle.
* The marker residue and desmethyldanofloxacin accounted for 10% and 90%, respectively, of the total residues in porcine liver.
* The marker residue accounted for 25% of the total residues in porcine kidney.
* The marker residue accounted for 90% of the total residues in chicken muscle.
* The marker residue and desmethyldanofloxacin accounted for 50% and 15%, respectively, of the total residues in chicken liver.
Toxicological data

At its present meeting, the Committee considered data from published references and unpublished field reports on the reproductive toxicity of dihydrostreptomycin and streptomycin. Although none of the studies or reports met current quality assurance standards, they did provide some useful information for evaluation of the safety of the compounds.

The field reports consisted of empirical observations by veterinarians and evaluations of fertility records for cattle, pigs, sheep and horses treated parenterally with streptomycin or a combination of dihydrostreptomycin and streptomycin for the treatment or control of leptospirosis. In most cases, the treatment consisted of two injections of 25 mg per kg of body weight of streptomycin given 14 days apart. Treatment regimens for cattle and horses consisting of daily injections of 5 g of streptomycin or of the combination for 3–5 consecutive days were also reported. None of these reports indicated that treatment of animals with streptomycin or the combination had any adverse effects on their fertility or reproductive performance.

The Committee was also asked to consider European directives that require bulls and boars to be injected with streptomycin for the control of *Leptospira* spp. upon entry to artificial insemination facilities. The treatment regimens consisted of two injections of streptomycin or of a combination of dihydrostreptomycin and streptomycin 14 days apart at a dose of 25 mg per kg of body weight. In one country alone, where 1500 bulls were treated over the course of 1 year, no adverse effects on the quality of semen produced were reported. The Committee recognized that the fertility of animals in artificial insemination centres is carefully monitored and therefore any adverse effects of streptomycin on semen quality would probably have been reported.

In a controlled study, nine bulls were injected subcutaneously with two doses of 22 mg per kg of body weight of dihydrostreptomycin administered 12 hours apart. Semen was collected and evaluated 3 and 7 days after treatment, and the results were compared with those for samples collected 5 days and 1 day before treatment. Semen samples were evaluated for pH, volume, percentage of motile spermatozoa, rate of spermatozoal motility, concentration of spermatozoa, and capacity to withstand freezing. Because it takes about 8 days for bovine sperm to pass through the epididymis, these post-treatment samples represented sperm that were in the epididymis at the time of treatment. On each of days 8, 17 and 26 after treatment,
one treated and one control bull underwent surgical removal of the testes. Testicular size and weight were recorded and all stages of spermatogenesis were examined histologically. The results showed no treatment-related effects on any of the parameters evaluated. The Committee concluded that treatment with streptomycin had no adverse effects on spermatogenesis in the bulls in this study.

The Committee reviewed new information on the pregnancy outcomes of women treated parenterally with streptomycin for tuberculosis. The new information supported the conclusion that streptomycin damages the eighth cranial nerve in offspring born to treated mothers. Rigorous literature searches revealed no other reported adverse reproductive effect associated with the use of streptomycin in humans.

At its forty-third meeting, the Committee evaluated a study of reproductive toxicity in ICR mice treated with 250mg of streptomycin per kg of body weight per day intraperitoneally on days 12–18 of gestation. Behavioural tests were conducted on the offspring. No treatment-related adverse effects on activity or functional development were observed. At its present meeting, the Committee concluded that, although the protocol of this study allowed an evaluation of prenatal effects, the results could not be used for evaluating perinatal or postnatal effects of streptomycin because the dams were not treated throughout lactation.

The Committee noted the long history of use of dihydrostreptomycin and streptomycin in veterinary and human medicine and the absence of reports in the literature of adverse effects on fertility or of perinatal or postnatal toxicity other than ototoxicity. Furthermore, the Committee recognized that very small amounts of aminoglycosides are absorbed after ingestion by adults and infants. It concluded that consumption of residues of dihydrostreptomycin or streptomycin in foods derived from animals treated in accordance with good practice in the use of veterinary drugs presents essentially no risk to perinatal or postnatal health in humans.

The Committee considered the potential risk to infants of consuming milk containing residues of dihydrostreptomycin or streptomycin during the first weeks of life, before closure of the gut. The temporary MRL for the combined residues of dihydrostreptomycin and streptomycin in cows' milk recommended at the forty-third meeting of the Committee was 200μg/l. In the toxicological monograph, the Committee noted that streptomycin is administered parenterally to infants for therapeutic purposes at doses of 10–20mg per kg of body weight per day (Annex 1, reference 114). At its present meeting, the Com-
mittee concluded that the low levels of these compounds permitted in cows’ milk present essentially no risk to newborns.

**Microbiological data**

At its forty-third meeting, the Committee determined that the ADI based on the antimicrobial activity of the combined residues of dihydrostreptomycin and streptomycin is 0–80μg per kg of body weight. The equation used by the Committee at its forty-third meeting was modified at the forty-seventh meeting (Annex 1, reference 125) by replacing the value for daily faecal bolus (150g) with a value for colonic content (220g). This increases the ADI based on the antimicrobial activity of the combined residues to 0–120μg per kg of body weight.

At its forty-third meeting, the Committee determined that the most sensitive effects caused by dihydrostreptomycin and streptomycin were those observed with dihydrostreptomycin in a 2-year oral toxicity study in rats. The NOEL was 5 mg per kg of body weight per day, based on the decrease in body-weight gain observed in males at the highest dose. At its present meeting, the Committee confirmed this as the appropriate NOEL. On the basis of this NOEL and using a safety factor of 100, the Committee established a group ADI of 0–50μg per kg of body weight for the combined residues of dihydrostreptomycin and streptomycin.

An addendum to the toxicological monograph was prepared.

**Residue data**

No new studies have been carried out on the metabolism of either streptomycin or dihydrostreptomycin. An evaluation report was submitted by the sponsor that provided the following information:

- Pharmacokinetic data from studies in animals and observations in humans given streptomycin and/or dihydrostreptomycin orally suggest that neither drug is metabolized to any significant extent.
- Aminoglycosides as a class of antimicrobials are not readily metabolized in humans. In particular, in studies using radiolabelled neomycin in cattle, no metabolites other than parent drug were identified in tissues or excreta.
- Neither streptomycin nor dihydrostreptomycin is likely to be absorbed from the gastrointestinal tract because of their high relative molecular mass and because they are excreted unchanged in faeces.
- When administered parenterally to humans, most of the streptomycin was excreted unchanged in the urine.
On the basis of the evaluation report, the Committee concluded that further information on metabolism was not required for the following reasons:

- Extrapolation from limited studies with other aminoglycosides in farm animals strongly suggests that neither streptomycin nor dihydrostreptomycin is metabolized in food-producing animals and humans.
- Additional studies may not yield substantial new information.

No new data were submitted on residues in eggs because the sponsor has not used and does not intend to use the drugs in laying birds. Accordingly, the Committee was unable to recommend an MRL for eggs.

**Analytical methods**

An HPLC method was developed for the measurement of dihydrostreptomycin in muscle, liver, kidney and fat of cattle, sheep and pigs. The extracts were analysed by HPLC with post-column derivatization and fluorescence detection. The method was evaluated using tissues fortified with dihydrostreptomycin at 400–5000 μg/kg. The method was linear over this range and the accuracy was acceptable. The limit of quantification was determined as 400 μg/kg for all tissues. There was no interference from streptomycin or procaine benzylpenicillin, but the specificity towards other antibiotics was not tested.

The HPLC method and an antimicrobial assay were compared using sheep liver and kidney tissues fortified with dihydrostreptomycin at 500 and 1200 μg/kg. The results for the two methods were equivalent. No comparison of the methods was made using residues in incurred tissues.

Cattle, sheep and pigs received three successive daily intramuscular injections of a preparation of dihydrostreptomycin combined with procaine benzylpenicillin at the recommended dose (10 mg per kg of body weight and 8 mg per kg of body weight, respectively). At 14 days or more post-treatment residues in the edible tissues of treated animals were below the limit of quantification of the HPLC method (400 μg/kg). Similar results were observed in studies submitted at the forty-third meeting (Annex 1, reference 113), in which an antimicrobial assay was used. Residues were found in tissues of cattle and sheep collected from the injection site at 14 and 18 days after treatment at the recommended dose, but were below the limit of quantification at 21 and 28 days after dosing, respectively. Residues at the injection site in cattle and sheep showed broad variability at 14 and 18 days following treatment. At 14 days after treatment, residues in cattle were
<400–982 µg/kg and at 18 days residues were <400–1140 µg/kg. In sheep, residues were 417–982 µg/kg at 14 days and <400–691 µg/kg at 18 days. No residues were found at the injection site in pigs killed at 14 and 18 days after dosing.

**Maximum Residue Limits**

- No MRLs can be recommended for eggs because no additional data were available on residues in eggs.
- The HPLC analytical method is satisfactory for monitoring the residues of dihydrostreptomycin but has not been tested for streptomycin. Therefore, the method cannot be recommended to regulate the MRL which is set as the sum of the two drugs. The Committee maintained the temporary MRLs recommended at its forty-third meeting (500 µg/kg for muscle, liver and fat and 1000 µg/kg for kidney in cattle, sheep, pigs and chickens, and 200 µg/l for milk in cattle, expressed as the sum of the concentrations of streptomycin and dihydrostreptomycin).

The following information is required for evaluation in 1999:

1. Validation of the HPLC assay to measure residues of streptomycin.
2. Studies to determine whether the HPLC and antimicrobial assay methods give similar results for residues of both drugs using tissues with incurred residues.

### 3.2.4 Enrofloxacín

Enrofloxacín is a fluoroquinolone antimicrobial agent that was evaluated at the forty-third meeting of the Committee (Annex 1, reference 113). At that time, a temporary ADI of 0–0.6 µg per kg of body weight was established, based on the results of the limited summary data on microbiological tests. The Committee requested the following information for evaluation in 1997:

1. Detailed reports of the *in vitro* MIC investigations of enrofloxacín that were submitted for evaluation.
2. Information on the effects of enrofloxacín and ciprofloxacín on specific genera of microorganisms obtained from the human intestine.

In addition, the Committee required that the results of studies to determine the antimicrobial activity of the residues other than enrofloxacín and ciprofloxacín be submitted for review as soon as they become available.
Toxicological and microbiological data

Because ciprofloxacin has been shown to be a main metabolite of enrofloxacin in some food-producing animals, the Committee considered new information on the pharmacokinetics of ciprofloxacin and its effects on the human intestinal microflora. New studies of the microbiological effects of enrofloxacin and ciprofloxacin in vitro were also evaluated by the Committee. All of these studies were conducted in accordance with appropriate standards for study protocol and conduct.

In humans 63–69% of an oral dose of ciprofloxacin is absorbed and this value is not significantly altered by administration with food. The main sites of absorption are the duodenum and jejunum. The recovery of high concentrations of ciprofloxacin in faeces is attributed primarily to secretion into the intestine. The Committee noted that fluoroquinolones as a class are active against aerobic Gram-negative bacteria. The primary clinical use of fluoroquinolones in humans is for selective elimination of potential aerobic and facultative anaerobic pathogens from the gastrointestinal tract while preserving the predominant anaerobic bacterial flora. These properties of the drug are clinically useful in the treatment of travellers’ diarrhoea, therapy for immunocompromised patients, selective decontamination prior to colorectal surgery, and therapy associated with burns and leukaemia. The Committee also noted that therapeutic doses of fluoroquinolones administered orally to humans have been shown to have no appreciable effect on the ecology of the human intestinal microflora and do not weaken the barrier effect. In addition, anaerobic bacteria such as Bifidobacterium spp., Bacteroides spp., Eubacterium spp., Fusobacterium spp. and Peptostreptococcus spp., the main components of the human gastrointestinal flora, are largely unaffected by these compounds. Finally, the Committee noted that, although Escherichia coli is extremely sensitive to fluoroquinolones in general, this species is a minor component of the gastrointestinal flora. These concepts guided the Committee in its evaluation and interpretation of the results of studies of the effects of enrofloxacin and ciprofloxacin on bacteria of the human intestinal flora in vitro and in vivo.

In two studies, the minimum concentrations of either enrofloxacin or ciprofloxacin causing 50% inhibition (MIC$_{50}$) were determined for 100 bacterial strains from 10 bacterial genera, many of which are typically found in the human gastrointestinal tract. These included Enterococcus spp., Escherichia coli, Lactobacillus spp., Proteus spp., Bacteroides spp., Bifidobacterium spp., Fusobacterium spp., Eubacterium spp., Peptostreptococcus spp. and Clostridium spp. In the first study, Escherichia coli was the most sensitive species to enrofloxacin,
with a mean MIC$_{50}$ value of 0.03 µg/ml at an inoculum density of 10$^7$ colony-forming units per ml. *Fusobacterium* was the most sensitive relevant genus, with a mean MIC$_{50}$ value of 0.125 µg/ml for the 10 strains isolated from the human gastrointestinal tract at an inoculum density of 10$^7$ colony-forming units per ml. In the second study, *E. coli* was the most sensitive species to ciprofloxacin, with a mean MIC$_{50}$ value of 0.016 µg/ml at an inoculum density of 10$^7$ colony-forming units per ml. *Bifidobacterium* was the most sensitive relevant genus, with a mean MIC$_{50}$ value of 0.031 µg/ml for the 10 strains isolated from the human gastrointestinal flora at an inoculum density of 10$^7$ colony-forming units per ml.

A study to determine the microbiological activity of enrofloxacin metabolites against aerobic bacteria was reviewed by the Committee. In this study, the MIC$_{50}$ values of enrofloxacin and nine of its metabolites were determined against 164 strains of aerobic bacteria isolated from the human intestinal microflora. Enrofloxacin and ciprofloxacin showed the most antimicrobial activity. *E. coli* was the most sensitive species tested, the mean MIC$_{50}$ values for enrofloxacin and ciprofloxacin against 33 strains of *E. coli* being 0.03 and 0.015 µg/ml, respectively, at an inoculum density of 10$^7$ colony-forming units per ml.

The effect of pH on the MIC$_{50}$ value of enrofloxacin against 36 bacterial isolates representative of the human intestinal flora was considered. The pH levels tested (7.2, 6.2 and 5.2) encompassed the range of conditions likely to occur in the lower regions of the human intestinal tract, while avoiding extremes likely to inhibit bacterial growth *in vitro*. The results were in agreement with those of previous studies with fluoroquinolones, which showed that antimicrobial activity decreased as the pH was lowered.

The effect of enrofloxacin on the growth of 10 bacterial strains isolated from the human gastrointestinal tract was evaluated after incubation in a simple *in vitro* model. Enrofloxacin was added to the test system at a concentration similar to the geometric mean MIC$_{50}$ previously determined for each microbial group and at 0.56 µg/ml, the concentration estimated to occur in the intestine after consumption of residues. The viable counts of all 10 strains exposed to enrofloxacin increased during the 18-hour incubation period; the growth of 8 of the 10 strains was comparable to that of controls.

Numerous reports on the effects of oral doses of ciprofloxacin on the intestinal flora of volunteers were available. The doses ranged from 50 mg twice daily to 750 mg three times daily. In general, the anaerobic bacteria were, at most, mildly suppressed. The aerobic
bacteria were the most sensitive to all doses of ciprofloxacin tested.

Three studies of the levels of ciprofloxacin in the faeces of volunteers who received oral doses of the compound enabled the Committee to obtain a more direct estimate of the concentration of the antimicrobial agent in the colon than from the commonly used indirect approach of subtracting the value for bioavailability from 1.0. In these studies, volunteers received daily oral doses of ciprofloxacin ranging from 100 to 1000 mg for up to 7 days. The concentrations of ciprofloxacin in the faeces varied widely between individuals. Two of the studies resulted in mean data that were used to estimate that approximately 20% of an oral dose of ciprofloxacin is present in the colon, assuming the mass of the colonic contents is 220 g. The Committee used this figure in establishing the upper limit of the ADI for the antimicrobial activity of enrofloxacin. This is in keeping with the decision taken at the forty-third meeting of the Committee to use data on excretion from studies of ciprofloxacin in humans for the purpose of calculating the upper limit of the ADI for the antimicrobial activity of enrofloxacin.

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

\[
\text{Upper limit of ADI} = \frac{0.125 \mu g/g^a \times 220 g^b}{0.20^c \times 1^d \times 60 kg^e}
\]

\[= 2.3 \mu g \text{ per kg of body weight}\]

The Committee noted that the antimicrobial activity of ciprofloxacin against the relevant human intestinal microflora was about four times that of enrofloxacin and that consumers may be exposed to residues of ciprofloxacin in some species of food-producing animals. The Committee therefore considered that the microbiological activity of ciprofloxacin should be taken into account in recommending MRLs for this metabolite.

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\(^a\) For the purpose of this evaluation, the MIC\(_{50}\) value is the mean MIC\(_{50}\) for enrofloxacin against the 10 strains of the most sensitive relevant genus isolated from the human intestinal tract, in this case Fusobacterium spp.

\(^b\) The mass of the colonic contents.

\(^c\) The fraction of the oral dose available to the gut microflora was based on a study in human volunteers, in which approximately 20% of an oral dose of ciprofloxacin was present in the colon.

\(^d\) A safety factor of 1 was used because extensive relevant microbiological data were available.

\(^e\) The weight of an adult person.
At its forty-third meeting, the Committee established a temporary ADI of 0–0.6 μg per kg of body weight on the basis of the limited summary data from microbiological tests on ciprofloxacin. At its present meeting, the Committee considered the toxicological data on enrofloxacin and the microbiological effects of enrofloxacin and ciprofloxacin and concluded that the microbiological data provided the most sensitive end-point for the establishment of an ADI. The Committee therefore established an ADI of 0–2 μg per kg of body weight for enrofloxacin, based on its antimicrobial activity against 10 strains of the most sensitive relevant genus isolated from the human gastrointestinal tract. The ADI was rounded to one significant figure, which is standard practice. The Committee noted that this ADI provided an adequate margin of safety in relation to the NOEL of 1.2 mg per kg of body weight per day for testicular toxicity in dogs described in the report of the forty-third meeting of the Committee.

An addendum to the toxicological monograph was prepared.

Residue data
The Committee postponed the evaluation of the residue data due to the late submission of important additional data.

3.2.5 Flumequine

Flumequine is a fluoroquinolone antimicrobial agent predominantly active against Gram-negative bacteria, which is used to control infections in beef cattle, sheep, pigs, chickens and farmed trout.

Flumequine was evaluated by the Committee at its forty-second meeting (Annex 1, reference 110). At that time, an ADI could not be established owing to the lack of adequate information on the toxicological and microbiological hazards of flumequine. At its forty-second meeting, the Committee requested that the following be supplied before it reviewed the compound again:

1. Further data from mice from which NOELs for hepatotoxicity could be identified.
2. Information on the tumorigenic mechanism of flumequine.
3. Further data relating to the possible induction of arthropathy by the compound.
4. Information on the microbiological safety of flumequine residues.
5. Appropriate residue data.

Toxicological data
Information from a range of studies on flumequine was available for assessment, including data on the induction of arthropathy in young
dogs, hepatotoxic and liver enzyme-inducing effects in mice, the possible mechanism of the hepatocarcinogenicity of flumequine and its effect on human gut microflora. The studies were carried out according to appropriate standards for study protocol and conduct.

In order to test the effects of flumequine on articular cartilage, it was administered as tablets to groups of 10 three-month-old beagle dogs at doses of 15, 30, 60 or 150mg per kg of body weight per day for 13 weeks; controls received tablets containing no drug. Four animals from each group were killed after 3 weeks. The animals showed no overt clinical signs of arthopathy. Gross necropsy revealed erosions of the joint surfaces in two of 10 dogs in the highest-dose group and in one of 10 animals in the lowest-dose group. Histopathological lesions of the articular cartilage were observed in one of 10 dogs given 60mg per kg of body weight per day and in three of 10 dogs given 150mg per kg of body weight per day. The severity of the lesions was similar at 3 and 13 weeks. The Committee considered that the gross lesions in the one animal at 15mg per kg of body weight per day were not treatment-related, since no histopathological alterations were found and no gross lesions were observed in dogs given doses of 30 or 60mg per kg of body weight per day. Therefore, the NOEL for induction of arthopathy in young dogs was 30mg per kg of body weight per day.

In a 13-week study designed to investigate hepatotoxic lesions and the activities of hepatic drug-metabolizing enzymes, flumequine was administered to male CD-1 mice in the feed at doses equal to 25, 50, 100, 400 or 800mg per kg of body weight per day and to females at 100, 400 or 800mg per kg of body weight per day; controls received feed containing no drug. The effects observed were reduced body weight, significantly increased plasma activities of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and lactate dehydrogenase, and increased liver weight at doses of 400 and 800mg per kg of body weight per day. Histopathological examination of the liver revealed dose-dependent hypertrophy, degenerative alterations and necrosis of the centrilobular hepatocytes. The hepatotoxic lesions were more pronounced in male than in female mice and were observed at all doses above 25mg per kg of body weight per day. Increased mitosis was observed only in males at a dose of 800mg per kg of body weight per day. Flumequine caused little or no induction of hepatic drug-metabolizing enzymes dependent on cytochrome P-450 and UDP glucuronosyltransferase when given at doses of up to 800mg per kg of body weight per day. The NOEL was 25mg per kg of body weight per day, based on the induction of hepatotoxic lesions in male mice.
At its forty-second meeting, the Committee noted that there was evidence of flumequine-related tumorigenic effects in the liver of CD-1 mice. The tumorigenic activity of flumequine was more pronounced in the livers of male mice, which are known to be sensitive to liver tumour induction. As the compound was inactive in a range of tests for genotoxicity, including in vitro assays for gene mutations in bacteria and mammalian cells and an in vivo assay for chromosomal aberrations in mammalian cells, the mechanism of tumorigenesis was unclear.

At its present meeting, the Committee noted that, although an inhibitory effect of flumequine on mammalian DNA gyrase, leading to DNA damage, was not investigated, bibliographical data on structurally related fluoroquinolones indicate that this mechanism is unlikely to operate. The Committee concluded that there is no evidence of any genotoxic potential of flumequine.

Non-genotoxic tumorigenesis in the liver can be due to various mechanisms, including treatment-related hormonal activity, proliferation of peroxisomes, induction of hepatic drug-metabolizing enzymes, or hepatotoxicity.

The toxicological database, including studies of reproductive toxicity, revealed no evidence of any hormonal activity of flumequine. In short-term and long-term toxicity studies in mice, no histopathological alterations of liver cells that indicate proliferation of peroxisomes were reported. Induction of the hepatic cytochrome P-450 enzyme system can be excluded on the basis of the results of the 13-week study in CD-1 mice.

Flumequine is hepatotoxic, causing hepatocellular degeneration and focal necrosis in male and female mice, which is followed by a mitogenic response in male mice at the highest dose in the 13-week study described above. The dose-related severity of these hepatotoxic lesions paralleled the incidence of benign and malignant liver tumours. Various non-genotoxic hepatotoxins have been shown to induce liver tumours. The mechanism is believed to be increased liver-cell proliferation due to repeated hepatocellular necrosis-regeneration cycles, leading to the development of foci of phenotypically altered hepatocytes (so-called “preneoplastic lesions”), which finally progress to neoplasms. In the 13-week study in groups of CD-1 mice (16 animals per group), clear-cell foci of altered hepatocytes, which are one type of preneoplastic lesion, were observed in one male that received 400 mg per kg of body weight per day of flumequine and in
one male and one female at a dose of 800 mg per kg of body weight per day.

In a special study to assess marker enzymes of hepatic preneoplastic lesions, the influence of flumequine on the activity of γ-glutamyltransferase and glutathione transferase was investigated in homogenates of livers from some of the mice used in the 13-week study. Because of its inadequate design, including the lack of histochemical characterization of the foci of altered hepatocytes, this study was not considered further in the evaluation.

The Committee considered that induction of hepatocellular necrosis-regeneration cycles by hepatotoxicity is the relevant mechanism for induction of liver tumours by flumequine. Therefore, the NOEL for the hepatotoxicity of flumequine, 25 mg per kg of body weight per day, was considered to be the threshold for both hepatotoxicity and its associated carcinogenicity. The Committee noted that hepatotoxicity would have been better explored in a study of longer duration.

Microbiological data
The effect of flumequine on human intestinal microflora was assessed by determining the MIC$_{50}$ values for 100 bacterial strains isolated from human faeces, comprising 10 isolates from 10 aerobic and anaerobic bacterial genera typical of the human gut microflora. These included *Escherichia coli*, *Streptococcus* spp., *Proteus* spp., *Lactobacillus* spp., *Bifidobacterium* spp., *Bacteroides* fragilis, *Eubacterium* spp., *Clostridium* spp., *Fusobacterium* spp. and *Peptostreptococcus* spp. The inoculum density was 10$^7$ colony-forming units per ml. *Escherichia coli* was the most sensitive bacterial species tested, with an MIC$_{50}$ value of 0.33 µg/ml. The MIC$_{50}$ value was not dependent on the size of the inoculum. *E. coli* was markedly less sensitive to 7-hydroxyflumequine, with an MIC$_{50}$ value of 4 µg/ml. *Fusobacterium* spp. and *Clostridium* spp. were the most sensitive relevant genera from the human gastrointestinal tract, with mean MIC$_{50}$ values of 1.0 and 0.95 µg/ml, respectively.

In a study investigating the influence of gastrointestinal factors and pH on the MIC$_{50}$ values of flumequine for relevant bacterial species of the human gastrointestinal tract, the MIC$_{50}$ values for obligate anaerobic bacteria were shown to be unaffected, whereas the mean MIC$_{50}$ values for *E. coli* were increased two- to eight-fold.

In calculating an ADI based on antimicrobial activity, the Committee used the formula described in section 3.2:
Upper limit of ADI = \(\frac{1\mu g/g^a \times 220g^b}{0.1^c \times 1^d \times 60kg^e}\)  
= 37\(\mu g\) per kg of body weight

The Committee noted that flumequine belongs to the group of antimicrobial fluoroquinolones that are active against aerobic Gram-negative bacteria. In humans, this class of antimicrobial agents is used clinically for selective elimination of potential aerobic and facultative anaerobic pathogens from the gastrointestinal tract while preserving the predominant anaerobic gut microflora. The Committee also recognized that administration of therapeutic oral doses of fluoroquinolones such as ciprofloxacin or norfloxacain to humans has no appreciable effect on the ecology of the intestinal flora or on the barrier effect. In addition, anaerobic bacteria such as *Bifidobacterium* spp., *Bacteroides* spp., *Eubacterium* spp., *Fusobacterium* spp. and *Peptostreptococcus* spp., the main components of the flora in the human gastrointestinal tract, are largely unaffected by these compounds. *Escherichia coli* is very sensitive to fluoroquinolones in general, but is a minor component of the gastrointestinal flora. On the basis of this information, the Committee considered that, in assessing the effects of flumequine on the human gastrointestinal flora, the MIC\(_{90}\) values for the selected bacterial species should be interpreted in the context of the overall ecosystem of the gastrointestinal tract. Since the obligate anaerobic bacteria that predominate in the gastrointestinal tract are relatively insensitive to fluoroquinolones, disturbance of the human gut ecosystem by residues of flumequine is unlikely. Therefore the Committee decided to base the ADI on the toxicological properties of flumequine and not on its effect on the intestinal microflora.

The Committee considered the NOEL of 25\(mg\) per kg of body weight per day for hepatotoxicity in the 13-week study in CD-1 mice to be the most appropriate toxicological end-point for consumer safety. It established an ADI of 0–30\(\mu g\) per kg of body weight based on this

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\(a\) The mean MIC\(_{90}\) for the most sensitive predominant bacterial species of the human gastrointestinal flora, in this case *Fusobacterium* spp. and *Clostridium* spp.

\(b\) The mass of the colonic contents.

\(c\) The fraction of the oral dose available to the gut microflora was based on a study in which 830\(mg\) of \(^{13}C\)flumequine was given orally to five healthy volunteers. The levels of radioactivity were then monitored in plasma, urine and faeces for up to 5 days. A total of 84\% (78–92\%) of the radioactivity was recovered in the excreta: 9\% (5.7–13\%) in faeces and 75\% (70–81\%) in urine. The Committee concluded that approximately 10\% of an oral dose of flumequine is available to the gut microflora.

\(d\) A safety factor of 1 was used because sufficient relevant microbiological data had been provided.

\(e\) The weight of an adult person.
NOEL and a safety factor of 1000, which was chosen to reflect the short duration of the study and the lack of histochemical characterization of the foci of altered hepatocytes. The ADI was rounded to one significant figure, which is the standard practice.

An addendum to the toxicological monograph was prepared.

Residue data

Flumequine is well absorbed when administered orally or parenterally and is excreted in the urine and faeces as the glucuronide conjugates of the parent drug and 7-hydroxyflumequine. In a study using \[^{14}\text{C}]\text{flumequine}\ in calves, 86–99% of the radioactivity in kidney, muscle and fat was extractable and the only metabolites found were flumequine and 7-hydroxyflumequine. Liver contained other unidentified residues, including a new metabolite (m1), which was the main metabolite found 24 hours after the last dose and at all subsequent time points. Flumequine accounted for 50–98% of the total residues in all edible tissues except liver, in which it accounted for approximately 25% of the total residues. The metabolite m1, which exhibited no antimicrobial activity, was present in both free and protein-bound fractions. The bound residues accounted for about 20% of total radioactivity in liver at all time points after 24 hours. The major residue found in the edible tissues of sheep, pigs and chickens was parent drug; there were also minor amounts of 7-hydroxyflumequine. The only residue detected in trout was the parent drug.

Flumequine was administered orally to 20 calves at an initial dose of 12 mg per kg of body weight, followed by nine doses of 6 mg per kg of body weight at 12-hourly intervals. The animals were killed in groups (four per group) at 24, 36, 48, 72 and 96 hours after the final dose and their tissues examined for flumequine residues. The highest mean residue levels were found in kidney (1.06 mg/kg) at 24 hours after the final dose, declining to 0.09 mg/kg at 96 hours. Residue levels in muscle declined from 0.35 mg/kg at 24 hours to 0.05 mg/kg at 96 hours, while levels in liver declined from 0.24 mg/kg at 24 hours to <0.018 mg/kg (limit of detection) at 96 hours and levels in fat declined from 0.19 mg/kg at 24 hours to <0.05 mg/kg (limit of quantification) at 72 hours.

In separate studies with the unlabelled drug, flumequine was administered orally to calves, sheep and pigs over 5 consecutive days. Calves and sheep received an initial dose of 12 mg per kg of body weight, followed by nine doses of 6 mg per kg of body weight at 12-hourly intervals. Pigs received an initial dose of 15 mg per kg of body weight, followed by nine doses of 7.5 mg per kg of body weight at intervals
Table 3
Maximum residue concentrations of flumequine in tissues of cattle and sheep given a single dose of 12mg per kg of body weight orally, followed by 9 doses of 6mg per kg of body weight at 12-hour intervals, and in tissues of pigs given a single dose of 15mg per kg of body weight orally, followed by 9 doses of 7.5mg per kg of body weight at 12-hour intervals

<table>
<thead>
<tr>
<th>Withdrawal time (h)</th>
<th>Residue concentration (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td><strong>Cattle</strong></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>0.43</td>
</tr>
<tr>
<td>36</td>
<td>0.18</td>
</tr>
<tr>
<td>48</td>
<td>0.16</td>
</tr>
<tr>
<td>72</td>
<td>0.17</td>
</tr>
<tr>
<td><strong>Sheep</strong></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>0.49</td>
</tr>
<tr>
<td>30</td>
<td>0.09</td>
</tr>
<tr>
<td>48</td>
<td>0.04</td>
</tr>
<tr>
<td>60</td>
<td>0.02</td>
</tr>
<tr>
<td>78</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Pigs</strong></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0.27</td>
</tr>
<tr>
<td>24</td>
<td>0.07</td>
</tr>
<tr>
<td>36</td>
<td>0.07</td>
</tr>
<tr>
<td>48</td>
<td>0.10</td>
</tr>
<tr>
<td>72</td>
<td>&lt;0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>96</td>
<td>&lt;0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Limit of quantification.

of 12 hours. The animals were killed in groups (four per group) at various times after the final dose and their tissues examined for flumequine residues. Table 3 shows the highest residue levels (corrected for recovery) found in the tissues at each time point.

Flumequine was administered ad lib in drinking-water to 36 broilers for 5 days at a dose equivalent to 12mg per kg of body weight per day. The broilers were killed in groups (six per group) at 6, 24, 36, 48, 72 and 96 hours after withdrawal of the drug. After 6 hours, the mean levels of flumequine residues in muscle, liver, kidney and fat/skin were 1.50, 2.46, 2.50 and 0.72mg/kg, respectively. By 48 hours after withdrawal of medication, the mean residue levels in these tissues had fallen to 0.07, 0.19, 0.16 and 0.08mg/kg, respectively, and at 96 hours flumequine could not be detected in any tissue analyses.

In a residue-depletion study in two groups of 200 trout maintained at about 7°C and 16°C, flumequine was administered as an oral premix at a rate of 6mg per kg of body weight every 12 hours for 5 days. Concentrations of flumequine were measured in muscle and skin mixed in natural proportions. At 1 day after the last drug treatment,
the maximum residue concentration of flumequine found in the tissues of the trout maintained at 7°C was 8.58 mg/kg, declining to 1.49 mg/kg at 4 days and to 0.13 mg/kg at 7 days. The maximum residue concentrations found in the trout maintained at 16°C were 3.65 mg/kg at 1 day following withdrawal of the drug and 0.08 mg/kg at 4 days. No residues of flumequine were detected at 14 days after withdrawal of medication in either group.

**Analytical methods**

An HPLC method for the determination of levels of residues of flumequine and its metabolite, 7-hydroxyflumequine, using fluorescence detection was used to obtain the data presented in the residue-depletion studies described above (see Table 3). Chromatography was performed after purification by liquid–liquid extraction. The method, which was used only in the sponsor's laboratory, was acceptable in terms of linearity, accuracy, repeatability and limits of quantification and detection. The method was specific for flumequine in the presence of a wide range of related quinolone antimicrobial agents. The limits of quantification were as follows: 0.1 mg/kg for liver and kidney of chickens; 0.05 mg/kg for liver, kidney and fat of calves, skin/fat of chickens, muscle/skin of trout and all edible tissues of pigs; 0.025 mg/kg for muscle of calves and chickens; and 0.005 mg/kg for all edible tissues of sheep.

**Maximum Residue Limits**

In reaching its decision on MRLs for flumequine, the Committee took the following factors into consideration:

- An ADI of 0–30 μg per kg of body weight, based on a toxicological-end-point, was established. This corresponds to a maximum ADI of 1800 μg for a 60-kg person.
- The parent drug was selected as the marker residue.
- Muscle and kidney were considered to be the appropriate target tissues. For practical reasons, however, liver is recommended as the target tissue for chickens in place of kidney.
- On the basis of data from studies in calves, non-extractable residues accounted for 20% of the total residues.
- In calf muscle, kidney and fat, the parent drug accounted for approximately 80% of the extractable residues.
- 7-Hydroxyflumequine and the unknown metabolite, m1, together account for 80% of the total radioactivity in calf liver after 168 hours.
- No data were provided on the amount of parent drug as a percentage of the total residues in sheep, chickens, pigs or trout.
Table 4
Theoretical maximum daily intake of flumequine residues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Recommended MRL (µg/kg)</th>
<th>Estimate of total residues (µg/kg)</th>
<th>Theoretical maximum daily intake (µg flumequine equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>500</td>
<td>1000</td>
<td>300</td>
</tr>
<tr>
<td>Liver</td>
<td>1000</td>
<td>4000</td>
<td>400</td>
</tr>
<tr>
<td>Kidney</td>
<td>3000</td>
<td>6000</td>
<td>300</td>
</tr>
<tr>
<td>Fat</td>
<td>1000</td>
<td>2000</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>1100</td>
</tr>
</tbody>
</table>

*Expressed as parent drug.*

*Based on a daily intake of 0.5 kg of meat made up of 300 g of muscle, 100 g of liver, and 50 g each of kidney and fat.*

*The marker residue accounted for 50% of the total residues in calf muscle, kidney and fat.*

*The marker residue accounted for 25% of the total residues in calf liver.*

- On the basis of the contribution of parent drug in extractable residues in calves, the parent drug accounts for 50% of the total residues in muscle, kidney and fat, and 25% of the total residues in liver.

- No data were provided for milk or eggs and no MRLs are recommended.

The Committee recommended MRLs for flumequine of 500 µg/kg for muscle, 1000 µg/kg for liver, 3000 µg/kg for kidney and 1000 µg/kg for fat in cattle, expressed as parent drug.

From these values for the MRLs, the maximum theoretical intake would be 1100 µg per day (Table 4). This would be compatible with a maximum ADI of 1800 µg for a 60-kg person.

In the absence of data on the contribution of parent drug to the total residues in sheep, chickens and pigs, the Committee recommended temporary MRLs of 500 µg/kg for muscle, 1000 µg/kg for liver, 3000 µg/kg for kidney and 1000 µg/kg for fat, expressed as parent drug, in these species. The Committee also recommended a temporary MRL of 500 µg/kg for trout muscle (including normal proportions of skin), expressed as parent drug.

The Committee requested that studies be conducted with radio-labelled flumequine in pigs, sheep, chickens and trout to estimate the proportion of the total residues accounted for by the parent drug. The results of these studies are required for evaluation in 2000.

3.2.6 Gentamicin

Gentamicin was evaluated at the forty-third meeting of the Committee (Annex 1, reference 113), when a temporary ADI of 0–4 µg per kg
of body weight was established. At that time, the Committee recommended temporary MRLs of 100 μg/kg for muscle and fat, 200 μg/kg for liver, and 1000 μg/kg for kidney in both cattle and pigs, as well as 100 μg/l for cows’ milk, all of the values being expressed as parent drug.

At its forty-third meeting, the Committee requested the following information for evaluation in 1997:

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

The results of these investigations were not available at the present meeting. However, the Committee was informed that the necessary studies are under way and the results will be available in the near future. Therefore, the Committee extended the temporary ADI and temporary MRLs for gentamicin until 1998.

3.2.7 Spiramycin

Residue data

Spiramycin had previously been evaluated at the twelfth, thirty-eighth, forty-third and forty-seventh meetings of the Committee (Annex 1, references 17, 97, 113 and 125). At its forty-third meeting, the Committee established an ADI of 0-50 μg per kg of body weight. It recommended MRLs for cattle (100 μg/kg for muscle, 300 μg/kg for liver and fat, 200 μg/kg for kidney and 100 μg/l for milk) and chickens (200 μg/kg for muscle, 400 μg/kg for liver, 800 μg/kg for kidney and 300 μg/kg for fat), expressed as the sum of spiramycin and neospiramycin concentrations. It also recommended MRLs for pigs of 200 μg/kg for muscle and fat, 600 μg/kg for liver and 300 μg/kg for kidney, expressed as spiramycin equivalents; the MRLs were temporary for pig liver, kidney and fat. The following information was requested 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 total antimicrobial activity accounted for by spiramycin and neospiramycin in the liver, kidney and fat of pigs.

At its forty-seventh meeting (Annex 1, reference 125), the Committee recommended MRLs for spiramycin of 200 µg/kg for muscle, 600 µg/kg for liver and 300 µg/kg for fat in cattle, pigs and chickens. It also recommended MRLs of 300 µg/kg for kidney in cattle and pigs and 800 µg/kg for chicken kidney, as well as 100 µg/l for cows’ milk. The MRLs are expressed as the sum of spiramycin and neospiramycin concentrations for cattle and chickens and as spiramycin equivalents for pigs.

At its Tenth Session (2), the Codex Committee on Residues of Veterinary Drugs in Foods considered the MRLs for spiramycin that were recommended at the forty-seventh meeting of the Expert Committee (Annex 1, reference 125). It requested that the Expert Committee reconsider the MRL for cows’ milk to determine whether it could be raised from 100 µg/l to 200 µg/l.

In response to the Codex Committee’s request, the Expert Committee reconsidered the MRLs for spiramycin at its present meeting. The MRLs recommended at the forty-seventh meeting of the Expert Committee (Annex 1, reference 125) result in a theoretical maximum daily intake of 440 µg of spiramycin residues (see Table 5). This is equivalent to 15% of the maximum ADI of 3000 µg of spiramycin residues for a 60-kg person. If the MRL for milk were increased to 200 µg/l, the theoretical maximum daily intake would increase to 590 µg or 20% of the maximum ADI.

<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 spiramycin equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>200</td>
<td>200</td>
<td>60</td>
</tr>
<tr>
<td>Liver</td>
<td>600</td>
<td>1200</td>
<td>120</td>
</tr>
<tr>
<td>Kidney (chickens)</td>
<td>800</td>
<td>1600</td>
<td>80</td>
</tr>
<tr>
<td>Fat</td>
<td>300</td>
<td>600</td>
<td>30</td>
</tr>
<tr>
<td>Milk</td>
<td>100</td>
<td>100</td>
<td>150</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>440</td>
</tr>
</tbody>
</table>

* Expressed as the sum of the concentrations of spiramycin and neospiramycin.
* Based on a daily intake of 300 g of muscle, 100 g of liver, 50 g of each kidney and fat, and 1.5 l of milk.
* Spiramycin and neospiramycin accounted for 100% of the total residues in muscle and milk.
* Spiramycin and neospiramycin accounted for 50% of the total residues in liver, kidney and fat.
Maximum Residue Limits
The Committee recommended that the MRL for cows' milk be increased from 100μg/l to 200μg/l.

3.3 Glucocorticosteroids
3.3.1 Dexamethasone
Dexamethasone was evaluated at the forty-second and forty-third meetings of the Committee (Annex 1, references 110 and 113). At its forty-second meeting, the Committee established an ADI of 0–0.015μg per kg of body weight and recommended temporary MRLs of 0.5μg/kg for muscle and kidney and 2.5μg/kg for liver in cattle and pigs, as well as 0.3μg/l for cows' milk, expressed as parent drug. Suitable methods for use in regulatory monitoring in all tissues at these MRLs were required for review in 1996.

At its forty-third meeting, the Committee recommended temporary MRLs for horses of 0.5μg/kg for muscle, 2.5μg/kg for liver and 0.5μg/kg for kidney, expressed as parent drug.

Data were not made available at the present meeting, although the Committee was aware that an analytical method and its performance characteristics were available to other organizations. Therefore, the temporary MRLs were not extended.

3.4 Insecticides
3.4.1 Cyfluthrin
Cyfluthrin has not been previously evaluated by the Committee. It was evaluated by the 1987 Joint FAO/WHO Meeting on Pesticide Residues (4), which established an ADI of 0–20μg per kg of body weight.

Cyfluthrin is an α-cyano type II pyrethroid insecticide, which is a mixture of four enantiomeric pairs of diastereoisomers resulting in eight optical isomers. It is manufactured at a typical purity of 92% (sum of all isomers) with six identified chemical impurities. Cyfluthrin formulations are applied topically and as sprays, dusts and fogs for the protection of cattle from infestation by flies and lice. The present submission is for a pour-on formulation.

Toxicological data
The Committee considered the data evaluated by the 1987 Joint FAO/WHO Meeting on Pesticide Residues, together with the results of several new studies. The studies were performed according to appropriate standards for study protocol and conduct.

In rats, cyfluthrin is readily absorbed and distributed. About 98% of the radiolabelled drug was eliminated in urine and faeces within 48
hours after oral administration; similar amounts (93–95%) were eliminated after intravenous administration. Orally administered cyfluthrin was also readily absorbed and distributed in lactating dairy cattle. The concentration of cyfluthrin in milk reached a maximum 3 days after initial dosing and then declined steadily. In cattle, the liver, kidney and fat contained the highest levels of residues, primarily of unmetabolized parent compound. The major metabolic transformation is ester hydrolysis to a 3-phenoxy-4-fluorobenzyl alcohol intermediate and a permethric acid moiety. After ester hydrolysis, the benzyl alcohol moiety is oxidized to the free 3-phenoxy-4-fluorobenzoic acid metabolite, which can be either conjugated with glycine or oxidized to give 4'-hydroxy-3-phenoxy-4-fluorobenzoic acid.

In rats, the oral LD$_{50}$ ranged from 16 to >1000mg per kg of body weight, depending on the vehicle used. At lethal or near lethal doses, the signs were typical of type II pyrethroids (including increased salivation, ataxia and reduced, laboured breathing). WHO has classified this substance as “moderately hazardous” (5).

Several short-term toxicity studies with cyfluthrin were available, including 4-week and 3-month studies in rats, a 4-week study in mice and a 6-month study in dogs. The dose levels ranged from 5 to 450mg per kg of body weight per day in all studies.

In these studies, cyfluthrin caused decreases in body-weight gain and increases in liver and kidney weights. At lethal or near lethal doses signs of neurotoxicity were observed in all species, including ataxia, abnormal gait and increased vocalization. Histological evidence of limited axonal demyelination of the sciatic nerves was also observed at lethal or near lethal doses, which was completely reversed within 3 months of cessation of treatment. The NOEL in rats ranged from 5mg per kg of body weight per day on the basis of depressed blood glucose levels, to 20mg per kg of body weight per day on the basis of mortality and decreased body-weight gain. The NOEL in mice was 45mg per kg of body weight per day on the basis of swelling of the glandular epithelium of the submaxillary salivary gland. The NOEL in dogs was equivalent to 2mg per kg of body weight per day, on the basis of lowered mean body weights.

In a 3-week toxicity study, rabbits received no drug or cyfluthrin by dermal application at 50 or 250mg per kg of body weight per day (in polyethylene glycol 400) for 6 hours per day on 5 consecutive days per week for 3 weeks. No toxicological effects were observed at any dose.

In another study in which dogs received no drug or cyfluthrin in the diet at up to 640mg/kg of feed (equal to 23mg per kg of body weight)
per day for 1 year, two out of six dogs at the highest dose exhibited slight disturbances in movement and gait on one occasion. There was no histopathological evidence of neurotoxicity. Slight increases in the weights of the spleens of females were observed at the highest dose. The NOEL was 160mg/kg of feed, equal to 5.1mg per kg of body weight per day.

In long-term toxicity and carcinogenicity studies, rats and mice received cyfluthrin or no drug in the diet. Rats were fed concentrations of up to 450 mg/kg of feed (equal to 19 mg per kg of body weight per day) and mice received 800 mg/kg of feed (equivalent to 120 mg per kg of body weight per day). The toxic effects were largely non-specific and were essentially restricted to minor alterations in body weight, organ weight and blood biochemical parameters. The NOELs were 50 mg/kg of feed in rats, equal to 2 mg per kg of body weight per day, and 200 mg/kg of feed in mice, equivalent to 30 mg/kg of body weight per day, both on the basis of decreased body-weight gain. Cyfluthrin treatment was not associated with increased tumorigenesis in either rats or mice.

In a 3-generation study of reproductive toxicity in rats, administration of cyfluthrin in the diet at 50, 150 or 450mg/kg of feed had no consistent effect on birth weight or litter size. A decrease in viability during lactation was noted in animals of the third generation at doses of 150 or 450mg/kg of feed, and weight gain was consistently lower in these animals than in untreated controls. The NOEL was 50mg/kg of feed, equivalent to 2.5mg per kg of body weight per day, on the basis of body-weight depression.

In a study of developmental toxicity, cyfluthrin was neither embryotoxic nor teratogenic in rats given doses of up to 30mg per kg of body weight per day on days 6–15 of gestation. The NOEL was 3mg per kg of body weight per day for maternal toxicity on the basis of clinical signs of altered gait and was 30mg per kg of body weight per day (the highest dose tested) for developmental effects. The developmental toxicity of cyfluthrin has also been evaluated in rabbits which received no drug or doses of 20, 60 or 180mg per kg of body weight per day by gavage on days 6–18 of gestation. Food consumption and body-weight gain were reduced and a dose-related increase in post-implantation losses was observed at doses of 60 and 180mg per kg of body weight per day. The NOEL for maternal toxicity and embryotoxicity was 20mg per kg of body weight per day on the basis of post-implantation losses at higher doses. The NOEL for developmental toxicity was 180mg per kg of body weight per day, the highest dose tested.
Cyfluthrin has been tested for its ability to induce DNA damage, chromosomal aberrations and gene mutations in vitro and chromosomal aberrations in vivo. Negative results were obtained in all of these studies. These results, in conjunction with those of the studies of carcinogenicity in rodents, indicate that cyfluthrin is neither genotoxic nor carcinogenic.

In studies of the neurotoxicity of cyfluthrin in rats, daily oral doses of 30–80mg per kg of body weight in polyethylene glycol 400 for up to 35 weeks resulted in characteristic signs of acute toxicity, including salivation, tremor and abnormal gait. Histological evidence of limited swelling and fragmentation of myelin was observed only infrequently, and these signs were completely reversible within 3 months of cessation of treatment. Rats were also evaluated in a neurobehavioural test, the inclined plane test, after administration of single oral doses of up to 0.1mg per kg of body weight of cyfluthrin in polyethoxylated castor oil. The results were inconsistent and not dose-dependent. Hence, the Committee considered that the results of this test were not useful for the assessment of neurotoxicity of this compound.

Although cyfluthrin is not an organophosphorus compound, it was also evaluated for its ability to induce delayed-type neurotoxicity in several studies in adult hens given single or multiple oral treatments of up to 5000mg per kg of body weight. The only neurological effects observed were acute behavioural disturbances and minor changes in sciatic nerves, including proliferation of Schwann cells and vacuolization of the myelin sheath. Cyfluthrin produced no symptoms of delayed-type neurotoxicity and no inhibition of neuropathy target esterase in brain, spinal cord or the peripheral nervous system.

The Committee concluded that cyfluthrin does not cause irreversible neurological damage and that the observed effects on the nervous system occur only at high doses.

The Committee concluded that the effects most relevant for the toxicological evaluation of cyfluthrin were those observed in the long-term study in rats fed the compound in the diet. The NOEL was 50mg/kg of feed, equal to 2mg per kg of body weight per day, on the basis of depression of body weight gain. Using this NOEL and a safety factor of 100, the Committee established an ADI for cyfluthrin of 0–20μg per kg of body weight. The Committee noted that the same value had been established by the 1987 Joint FAO/WHO Meeting on Pesticide Residues.

A toxicological monograph was prepared.
Residue data

The Committee reviewed numerous data, including proprietary information and data from the literature. A comprehensive expert report covering the use of cyfluthrin in veterinary medicine and a report dealing with the residues of the compound that are likely to result from non-veterinary uses were also reviewed.

Residue-depletion studies with radiolabelled cyfluthrin were not available for the present review. All studies presented were conducted with unlabelled cyfluthrin in a variety of formulations.

In three similarly-designed residue-depletion studies, a formulation of cyfluthrin was topically applied to cattle at dose levels of 400mg per animal (0.9–1.1mg per kg of body weight) or 800mg per animal (1.8–2.3mg per kg of body weight). The formulation was prepared from a 10% wettable powder suspended in water. The animals were killed in groups (three per group) on days 1, 3 and 7 in the first study, on days 10 and 14 in the second study, and on day 42 in the third study. None of the samples of muscle, liver or kidney contained detectable residues of the parent compound. The limit of detection was 10μg/kg. The highest concentrations were found in samples of visceral fat 14 days after treatment: 70–100μg/kg in cattle treated with 1.8–2.3mg per kg of body weight and 30–90μg/kg in cattle treated with 0.9–1.1mg per kg of body weight.

In four other studies, cattle were dosed with 1% pour-on formulations of cyfluthrin. In the first of these studies, 21 cattle each received a single dose of 200mg (0.5–0.93mg per kg of body weight). Three animals were slaughtered 1, 3, 7, 10, 14, 21 and 28 days after treatment. Fat was the only tissue that contained quantifiable residues. The highest concentrations, approximately 50μg/kg, were found 7, 10 and 14 days after treatment.

In the second study, cattle were treated with cyfluthrin at a dose of 0.33mg per kg of body weight. Two animals were killed 1, 4, 7, 14, 21 and 28 days after treatment. No detectable residues were present in muscle, liver or kidney. The concentrations of residues in fat increased from 14 and 15μg/kg on day 1 to a maximum of 32 and 36μg/kg on day 14 and then decreased to 14 and 18μg/kg 28 days after treatment.

In the third study, two groups of three cattle each were treated. One group received 0.63 mg per kg of body weight on day 1 and a second dose of 0.9 mg per kg of body weight 13 days later. The animals were killed 2 days after the second treatment. No residues were detected in muscle. Residues of the parent compound were found in liver (6–9 μg/kg), kidney (11–13 μg/kg) and fat (72–110 μg/kg). The other group
was treated five times with 0.9 mg per kg of body weight (one daily dose for the first 3 days, followed by two further doses at 12-day intervals). The animals were killed 2 days after the final dose. The concentrations of residues were 8–22 µg/kg in liver, 12–23 µg/kg in kidney and 86–240 µg/kg in fat. This study showed that under the conditions described, which represented a significant overdose, residue levels in fat were about 7.5 times those in kidney and 9.5 times those in liver.

In the fourth study, three groups each of three yearling cattle were treated with either one, two or three doses of cyfluthrin at a dose of 0.44 mg per kg of body weight. The interval between doses was 21 days. Animals were slaughtered 3 days after the final dose. Fat was the only tissue in which quantifiable residues were found. The concentrations of residues increased with the number of repeated doses and were 21–42 µg/kg after one dose, 77–102 µg/kg after two doses and 104–151 µg/kg after three doses.

The above four studies indicate that residues in fat increase with increasing dosage and only in the third study, in which animals received significant overdoses, were residues quantifiable in liver and kidney. No residues were detected in muscle tissue at any dose.

In a study to determine the level of residues in milk, two groups of three dairy cattle each were treated with a 1% pour-on formulation of cyfluthrin. The first group received a single dose equivalent to 0.63 mg per kg of body weight. In the second group the animals were treated with 0.9 mg per kg of body weight daily for 3 consecutive days. In animals that received a single dose the maximum concentrations of the parent drug in whole milk (6–26 µg/kg) were found 2–4 days after treatment. At 7 days after treatment the concentrations of residues were 2–5 µg/kg. In milk samples from the animals treated with three doses of 0.9 mg per kg of body weight, maximum concentrations were reached 1–2 days after the final dose and ranged from 23 to 54 µg/kg.

**Analytical methods**

A routine method for the determination of the marker residue of cyfluthrin in bovine tissues and milk was proposed. The residues are extracted with organic solvent and solid-phase chromatography and subjected to capillary gas–liquid chromatography with electron-capture detection. The four diastereoisomers are sufficiently resolved to allow their separate determination.

Recovery experiments were conducted with tissues fortified at two concentrations (10 and 50 µg/kg for muscle, liver, kidney and fat;
5 and 50μg/l for milk). Depending on the matrix and the analyte concentration, the recoveries were between 72 and 89%, with relative standard deviations of 4–12%.

The reported limits of detection of the method were 1μg/l for milk, 2μg/kg for muscle and kidney, and 3μg/kg for liver and fat. The limit of quantification was 5μg/l for milk and 10μg/kg for muscle, liver, kidney and fat. The specificity of the method was confirmed in the presence of permethrin and cypermethrin.

**Maximum Residue Limits**

The Committee confirmed the ADI of 0–20μg per kg of body weight previously allocated by the 1987 Joint FAO/WHO Meeting on Pesticide Residues. This would result in a maximum ADI of 1200μg for a 60-kg person.

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

- Fat was the only suitable target tissue.
- The concentrations of residue in liver and kidney are about one-tenth of those in fat.
- The parent compound was the marker residue and the only residue of concern.
- None of the available residue studies had been conducted exactly at the upper limit of the recommended dose range (0.5 mg per kg of body weight) and with repeated dosing at 28-day intervals, the shortest recommended time interval for repetition of treatment. The available data showed that:
  
  — estimates of the maximum residue concentrations resulting from a given single dose could be obtained by linear interpolation of the data obtained with other similar doses;
  
  — only studies with identical formulations of cyfluthrin could be compared quantitatively;
  
  — the kinetics of repeated treatment could be described by superimposing the data obtained from single-dose treatments with the same formulation;
  
  — the results obtained after identical treatment could vary greatly from animal to animal.

The maximum residue concentration in milk, expressed as the geometric mean of the data, could be interpolated to approximately 13 μg/l. The expected maximum residue level in fat at the dose of 0.5 mg per kg of body weight, which was the upper limit of the recommended dose range, could be estimated by interpolation from the results of the available studies to be 40 μg/kg.
The maximum residue concentrations in fat were reached between 10 and 14 days after a single dose. The residue levels decreased to 50% at approximately 28 days after treatment and to 30% of the maximum concentration by about 40 days after treatment. The expected numerical value of the maximum residue concentration in fat after treatment had been repeated once would be approximately 1.3 times the maximum value obtained after a single dose. If the treatment is repeated more than once at 28-day intervals, the maximum residue concentration would probably not exceed 1.5 times the maximum value obtained after a single dose. It could therefore be estimated that the highest concentration, expressed as the geometric mean of the obtained values, likely to be observed after repeated treatments at the recommended dose interval and the upper limit of the recommended dose range would be approximately $1.5 \times 40 \, \mu\text{g/kg} = 60 \, \mu\text{g/kg}$. The Committee considered that this value could serve as a basis for establishing an MRL in fat.

It was, however, also necessary to consider the significant biological variability of the residue concentrations in fat of individual animals. It was observed that the upper 99% confidence limit would cover this variability.

The Committee recommended an MRL for cyfluthrin of 200$\mu$g/kg for fat. This is the only MRL which can be used to monitor residues of cyfluthrin in the edible tissues of treated cattle. The recommended MRL for both liver and kidney was 20$\mu$g/kg. The recommended MRL for muscle was 20$\mu$g/kg on the basis of twice the limit of quantification of the analytical method. This MRL, however, far exceeds the estimated concentrations of the residues in muscle that would result from the use of the drug according to the manufacturer’s instructions and therefore cannot be used to monitor residues in the other tissues. Liver and kidney tissues are not suited as target tissues for the control of residues of cyfluthrin in cattle. An MRL of 40$\mu$g/l was recommended for milk, taking into account the standard deviation of the maximum residue concentration in milk.

The MRLs recommended above would result in a theoretical maximum daily intake of 79$\mu$g of cyfluthrin, based on a daily food intake of 300g of muscle, 100g of liver, 50g each of kidney and fat, and 1.5l of milk (Annex 1, reference 85). This is equivalent to 6.6% of the maximum permitted ADI.

The Committee noted that the MRLs for apple, cotton seed, rape seed, maize, sweet peppers and tomato recommended by the 1987 Joint FAO/WHO Meeting on Pesticide Residues resulted in a
theoretical maximum daily intake of 4% of the maximum ADI for a Middle Eastern diet, 0.7% for a Far Eastern diet, 1.2% for an African diet, 1.5% for a Latin American diet and 4.6% for a European diet, respectively. It also noted that the 1987 Joint FAO/WHO Meeting on Pesticide Residues had recommended an MRL of 10µg/l for milk, suggesting that it was covering the use of cyfluthrin as a veterinary drug. At its present meeting, however, the Committee noted that important studies of the residues in milk had not been available to the Joint FAO/WHO Meeting on Pesticide Residues for evaluation. It was, therefore, necessary to propose a higher MRL for milk, in order to cover the authorized uses of cyfluthrin in veterinary medicine.

3.4.2 Fluazuron

Fluazuron had not previously been considered by the Committee. It is an insect growth regulator that belongs to the class of benzoylphenyl urea derivatives which inhibit chitin synthesis. Fluazuron specifically interferes with chitin formation in ticks during engorgement, moulting and hatching. It is applied topically to beef cattle.

Toxicological data

The Committee considered the results of studies on the pharmacokinetics, metabolism, acute, short-term and long-term toxicity, carcinogenicity, genotoxicity and reproductive toxicity of fluazuron. All of the studies critical for the evaluation were carried out according to appropriate standards for study protocol and conduct.

Analysis 24 hours after administration of radiolabelled fluazuron to rats showed that an average of 60% of an oral dose was absorbed through the gut. Most of the absorbed radiolabel was taken up by the adipose tissues, while significantly lower levels were found in liver, kidney, lung, muscle and brain. Ninety per cent of the radiolabel in tissues was attached to unchanged fluazuron. Fluazuron was eliminated slowly from the adipose tissues, following first-order kinetics, with a half-life of about 13 days. Elimination occurred primarily in the faeces. One week after administration, 59% of the dose had been eliminated in faeces and 3% in urine. About one-third of the amount eliminated in faeces was unchanged fluazuron; the remainder was metabolized by cleavage of the urea moiety between the benzoyl carbon and the urea nitrogen followed by hydroxylation in the phenyl ring.

After topical administration of radiolabelled fluazuron to cattle, absorption occurred slowly, either percutaneously or orally (by licking) or by both routes. A steady state between absorption and elimination
was observed for 3–4 weeks after treatment. Most of the absorbed radiolabel was taken up by the adipose tissues and to a lesser extent by other tissues. Depletion of fluazuron from plasma and tissues was slow, with half-lives of elimination of 10.5 and 4.5–5.5 weeks, respectively. The major route of elimination was via the faeces (62% of the dose within 16 weeks), while urinary excretion was of minor importance (1% of the dose within 16 weeks). There was some indication of excretion in the bile. Fluazuron was not extensively metabolized as unchanged fluazuron accounted for more than 90% of the total residues in tissues and faeces. The pattern of absorption, distribution and elimination was similar after subcutaneous administration of radio-labelled fluazuron to steers. The pattern of metabolites excreted in faeces was somewhat more complex, however, indicating that about one-third of the fluazuron was metabolized into more polar metabolites. Although the fate of fluazuron in rats was similar to that in cattle, rats metabolized fluazuron to a greater extent than cattle.

Orally administered fluazuron was found to have low toxicity in an acute toxicity study in rats, with an LD₅₀ value of >5000mg per kg of body weight.

The short-term toxicity of fluazuron was evaluated from studies in which it was administered orally to rats and dogs. In a 28-day study, rats received no drug or fluazuron by gavage at doses of 10, 100 or 2000mg per kg of body weight per day. Increased prothrombin time and liver weight, and decreased platelet counts and thymus weight were observed. These effects occurred mainly in animals given doses of 100 or 2000mg per kg of body weight per day, particularly males. Male rats were also found to be more sensitive than females to the effects of fluazuron in a 13-week study in which rats were administered no drug or 100, 600, 3500 or 20000mg/kg of feed (equal to 6.4–1400mg per kg of body weight per day). Male rats given 3500 or 20000mg/kg of feed had increased prothrombin times, platelet and lymphocyte counts, and absolute and relative liver weights, as well as hypertrophy of thyroid follicular cells, pituitary cells and hepatocytes. In males, increases in absolute and relative liver weights were also observed at 600mg/kg of feed. In females, hepatocellular hypertrophy occurred at 3500 and 20000mg/kg of feed. The NOEL was 100mg/kg of feed, equal to 6.4mg per kg of body weight per day, on the basis of effects on the liver.

In a 1-year feeding study, dogs received no drug or fluazuron at dietary concentrations of 200, 3000 or 50000mg/kg of feed. Effects were observed primarily in males at the highest dose and consisted of decreased food consumption, transient loss of body weight, increased
serum activities of alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase, and minimal multifocal haemorrhage with slight multifocal chronic inflammation in the liver. A slight increase in alkaline phosphatase activity was also observed in males at 3000 mg/kg of feed and in females at 50000 mg/kg of feed. The NOEL was 200 mg/kg of feed, equal to 7.5 mg per kg of body weight per day, on the basis of changes in hepatic enzyme activities.

In a study of carcinogenicity, mice received no drug or fluazuron at 40, 400, 4000 or 9000 mg/kg of feed for 2 years (equal to 4.3–990 mg per kg of body weight per day). Females given 4000 or 9000 mg/kg of feed showed increased water consumption, cataracts, uterine changes (inflammatory polyps, dilatation of the uterine lumen) and, at 9000 mg/kg of feed only, haematomas and dilatation of blood vessels associated with thrombosis. Increased water consumption and inflammatory uterine polyps were also observed in females at 400 mg/kg of feed. At 4000 or 9000 mg/kg of feed male mice had cataracts, and a slightly increased frequency of diffuse hyperplasia of prostatic glandular tissue. The NOEL was 40 mg/kg of feed, equal to 4.3 mg per kg of body weight per day, on the basis of pathological changes in the uterus.

In a long-term study of toxicity and carcinogenicity, rats were given no drug or fluazuron at 50, 500, 10000 or 20000 mg/kg of feed for up to 2 years (equal to 1.9–920 mg per kg of body weight per day); a proportion of the animals were killed after 1 year. No toxicologically significant effects were observed at any dose. The toxic effects on the liver observed in the 13-week study in rats were not observed after either 1 year or 2 years.

In the long-term studies described above, the total amount of fluazuron in the body appeared to reach a threshold at relatively low levels (approximately 400 and 500 mg/kg of feed, equal to 43 and 18 mg per kg of body weight per day, in mice and rats, respectively), as the concentration of the compound in blood and fat did not increase at higher dose levels. The reason is not clear. It is not known whether a similar effect occurred in the short-term studies because they did not include measurements of fluazuron levels in blood and fat.

Fluazuron has been tested in vitro for its ability to induce reverse mutations in Salmonella typhimurium, gene mutations in Chinese hamster cells, chromosomal aberrations in human lymphocytes, and DNA repair in rat hepatocytes and human fibroblasts. It has also been tested in vivo for its ability to induce nuclear anomalies in Chinese hamster bone marrow. The results of all these tests were negative. On
the basis of these data and the results of the carcinogenicity studies in rodents, the Committee concluded that fluazuron has no genotoxic or carcinogenic potential.

In a 2-generation study of reproductive toxicity, rats received no drug or fluazuron at 100, 1500 or 20000 mg/kg of feed (equivalent to 5–1000 mg per kg of body weight per day). There was no evidence of adverse effects on reproductive function. The only effects observed were slightly retarded growth of pups at 1500 and 20000 mg/kg of feed and a slight increase in neonatal mortality at 20000 mg/kg of feed. The NOEL was 100 mg/kg of feed, equivalent to 5 mg per kg of body weight per day, on the basis of postnatal toxicity.

Fluazuron was not maternally toxic and there was no evidence of embryotoxicity, fetotoxicity or teratogenicity in rats or rabbits at oral doses of up to 1000 mg per kg of body weight per day.

Because fluazuron belongs to a class of insect growth regulators that do not act on the nervous system and because the central nervous system was not a target organ in the short-term and long-term studies of toxicity in rats, mice or dogs, special studies of neurotoxicity have not been performed. The Committee concluded that studies of neurotoxicity were not necessary.

The Committee established an ADI of 0–40 μg per kg of body weight for fluazuron, based on the NOEL of 4.3 mg per kg of body weight per day for pathological changes in the uterus in the 2-year study in mice and a safety factor of 100. The ADI was rounded to one significant figure, as is the usual practice.

A toxicological monograph was prepared.

*Metabolism data*

Rats received [14C]fluazuron by gavage at a dose of 0.5 mg per kg of body weight daily for 7 days. They were killed in groups (three males and three females per group) at 24 hours and 2, 4, 8 and 12 weeks after the final dose. In all organs and tissues examined approximately 90–100% of the radioactivity was extractable and unchanged fluazuron was the only detectable compound. Six distinct faecal metabolites were identified and eight metabolic fractions were separated from urine. The study indicates that fluazuron is metabolized slowly, but to a significant extent. About one-third of the eliminated radioactivity was in the form of unchanged parent drug; the remainder was metabolized, mainly via cleavage of the benzoyl-ureido bridge.

A study was conducted in Hereford steers, which were treated with a single subcutaneous injection of [14C]fluazuron at a dose of 1.5 mg per
Table 6
Residue levels of fluazuron (mg/kg) in tissues of cattle given a single subcutaneous injection of [14C]fluazuron at 1.5 mg per kg of body weight

<table>
<thead>
<tr>
<th>Withdrawal time</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 days</td>
<td>0.14</td>
<td>0.70</td>
<td>0.44</td>
<td>4.53</td>
</tr>
<tr>
<td>2 weeks</td>
<td>0.07</td>
<td>0.28</td>
<td>0.13</td>
<td>2.60</td>
</tr>
<tr>
<td>6 weeks</td>
<td>0.06</td>
<td>0.28</td>
<td>0.15</td>
<td>2.60</td>
</tr>
<tr>
<td>16 weeks</td>
<td>0.02</td>
<td>0.12</td>
<td>0.11</td>
<td>0.95</td>
</tr>
</tbody>
</table>

kg of body weight. The animals were killed in groups (three per group) at 2 days and 2, 6 and 16 weeks after treatment. In muscle, liver, kidney and fat, unchanged fluazuron was the only residue identified by thin layer chromatography, accounting for at least 80% of the total radioactive residues at all sampling times. The metabolites eliminated in faeces and urine were more polar than fluazuron at all sampling times; however, the extent of metabolism appeared to be lower in cattle than in rats.

Residue data
Table 6 shows the distribution of residues of fluazuron in the edible tissues of cattle in the study described above. Residue levels in fat were consistently about 10 times those in liver and kidney. Maximum residue levels were reached in all tissues at 2 days after drug withdrawal. Fat contained the highest amounts of residue at all sampling times.

Five residue-depletion studies in cattle treated with a pour-on formulation of fluazuron were considered. In the first and second studies, fluazuron was administered once, at a dose of 2 mg per kg of body weight (recommended dose) and 3 mg per kg of body weight, respectively. In the remaining studies, the drug was administered as follows: 2 mg per kg of body weight, repeated after 9 weeks; 3 mg per kg of body weight, repeated after 9 weeks; 2 or 4 mg per kg of body weight, repeated after 12 and 24 weeks. All these studies confirmed that the highest levels of residues occur in fat. Residue levels were similar from 6 to 16 weeks after treatment. Table 7 shows the distribution of residues in tissues of cattle given two doses of 3 mg per kg of body weight at an interval of 9 weeks. This dosing regimen exceeds that recommended for therapeutic use. The results of this study are consis-
Table 7
Residue levels of fluazuron (mg/kg) in tissues of three cattle given two pour-on applications of fluazuron at 3 mg per kg of body weight at an interval of 9 weeks

<table>
<thead>
<tr>
<th>Withdrawal time (weeks)</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>&lt;0.02-0.08</td>
<td>0.02-0.20</td>
<td>0.02-0.07</td>
<td>0.37-2.50</td>
</tr>
<tr>
<td>8</td>
<td>0.03</td>
<td>0.05-0.08</td>
<td>0.03</td>
<td>0.84-1.10</td>
</tr>
<tr>
<td>16</td>
<td>0.04-0.10</td>
<td>0.05-0.14</td>
<td>&lt;0.02-0.06</td>
<td>0.60-2.26</td>
</tr>
</tbody>
</table>

Tent with those of previous residue-depletion studies using multiple-dose regimens, in which there was no evidence of bioaccumulation of the drug, but residue levels were shown to increase in a dose-dependent manner.

Data on the depletion of fluazuron residues from fat from studies in cattle were evaluated. The data indicate that the residue concentrations of fluazuron in fat increase significantly in cattle as the body-fat content decreases. Therefore, these studies suggest that the MRLs for fat should be higher than those indicated by the results of the study using radiolabelled fluazuron or by the five residue-depletion studies.

**Analytical method**

Fluazuron residues can be measured by HPLC with fluorescence detection. The method has been used for the analysis of residues in tissues of animals following treatment with fluazuron as well as in tissues fortified with the drug. The method has limits of quantification of 0.02 mg/kg in muscle, liver and kidney and 0.01 mg/kg in fat. The overall mean recovery in all tissues was 95% (range 90–100%).

**Maximum Residue Limits**

In reaching its decision on MRLs for fluazuron, the Committee took into account the following factors:

- An ADI of 0–40 μg per kg of body weight was established. This would result in a maximum daily intake of 2400 μg for a 60-kg person.
- The marker residue is the parent drug.
- The parent drug accounts for at least 80% of the total residues in all tissues.
- Fat and liver or fat and kidney are recommended as the appropriate target tissues.
Table 8
Theoretical maximum daily intake of fluazuron residues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Recommended MRL (µg/kg)</th>
<th>Estimate of total residues (µg/kg)</th>
<th>Theoretical maximum daily intake (µg fluazuron equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>200</td>
<td>250&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75</td>
</tr>
<tr>
<td>Liver</td>
<td>500</td>
<td>625&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.5</td>
</tr>
<tr>
<td>Kidney</td>
<td>500</td>
<td>625&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.25</td>
</tr>
<tr>
<td>Fat</td>
<td>7000</td>
<td>8750&lt;sup&gt;c&lt;/sup&gt;</td>
<td>437.5</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>606.25</td>
</tr>
</tbody>
</table>

<sup>a</sup> Expressed as parent drug.
<sup>b</sup> Based on a daily intake of 0.5 kg of meat made up of 300 g of muscle, 100 g of liver, and 50 g each of kidney and fat.
<sup>c</sup> The marker residue accounted for 80% of the total residues in muscle, liver, kidney and fat.

- Suitable analytical methods are available for monitoring the proposed MRLs.

On the basis of the maximum observed residues in cattle treated with fluazuron in accordance with good practice in the use of veterinary drugs, the Committee recommended MRLs in cattle of 200 µg/kg for muscle, 500 µg/kg for liver and kidney and 7000 µg/kg for fat, expressed as parent drug. From these values, the theoretical maximum daily intake of fluazuron residues is 606 µg (Table 8).

4. **Recommendations**

1. Recommendations relating to specific veterinary drugs, including ADIs and MRLs, are given in section 3 and Annex 2.
2. In view of the large number of veterinary drugs requiring evaluation, meetings of the Joint FAO/WHO Expert Committee on Food Additives should be held annually for this purpose.
3. It was brought to the attention of the Committee that relevant data relating to the assessment of the effects of oxytetracycline on the human intestinal flora have become available since the previous toxicological evaluation of the substance at the thirty-sixth meeting in 1990 (Annex 1, reference 91). Therefore, the Committee recommended that oxytetracycline, chlortetracycline and tetracycline be re-evaluated at a future meeting.

**Acknowledgement**

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


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


4. *Specifications for identity and purity of food additives (food colours)* (Fourth report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as *Specifications for identity and purity of food additives*, vol. II. *Food colours*. Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).


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.


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

**Anthelminthic agents**

*Moxidectin*

ADI: 0–2μg 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); MRLs for sheep muscle were established at the forty-seventh meeting of the Committee (WHO Technical Report Series, No. 876, 1998)).

<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>20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sheep</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Deer</td>
<td>20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> 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 over a 49-day period after dosing.

<sup>b</sup> At its present meeting, the Committee reconsidered the MRL for cattle muscle, but on the basis of the available data decided to maintain the previous MRL.

<sup>c</sup> 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.

<sup>d</sup> 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).

**Tiabendazole (thiabendazole)**

ADI: 0–100μg per kg of body weight.

Residue definition: Sum of tiabendazole and 5-hydroxytiabendazole (MRLs were recommended at the fortieth meeting of the Committee (WHO Technical Report Series, No. 832, 1993)).
<table>
<thead>
<tr>
<th>Species</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
<th>Milk*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Pigs</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>Sheep</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>Goats</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

* Expressed in µg/l.

**Antimicrobial agents**

**Ceftiofur**

ADI: 0–50µg per kg of body weight (established at the forty-fifth meeting of the Committee (WHO Technical Report Series, No. 864, 1996)).

Residue definition: Desfuroylceftiofur (MRLs for cattle and pig liver and for cows’ milk were recommended 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>1000</td>
</tr>
<tr>
<td>Pigs</td>
<td>1000</td>
</tr>
</tbody>
</table>

* Expressed in µg/l.

**Danofloxacin**

ADI: 0–20µg per kg of body weight.

Residue definition: Danofloxacin.

<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</td>
</tr>
<tr>
<td>Pigs</td>
<td>100</td>
</tr>
<tr>
<td>Chickens</td>
<td>200</td>
</tr>
</tbody>
</table>

* Fat/skin in normal proportions.
Dihydrostreptomycin and streptomycin

ADI: 0–50 μg per kg of body weight (group ADI for the combined residues of dihydrostreptomycin and streptomycin).

Residue definition: Sum of the concentrations of dihydrostreptomycin and streptomycin (MRLs were recommended at the forty-third meeting of the Committee (WHO Technical Report Series, No. 855, 1995)).

<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>
<th>Eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>500&lt;sup&gt;b&lt;/sup&gt;</td>
<td>500&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1000&lt;sup&gt;c&lt;/sup&gt;</td>
<td>500&lt;sup&gt;c&lt;/sup&gt;</td>
<td>200&lt;sup&gt;b&lt;/sup&gt;</td>
<td>—</td>
</tr>
<tr>
<td>Pigs</td>
<td>500&lt;sup&gt;b&lt;/sup&gt;</td>
<td>500&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1000&lt;sup&gt;c&lt;/sup&gt;</td>
<td>500&lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Sheep</td>
<td>500&lt;sup&gt;b&lt;/sup&gt;</td>
<td>500&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1000&lt;sup&gt;c&lt;/sup&gt;</td>
<td>500&lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Chickens</td>
<td>500&lt;sup&gt;b&lt;/sup&gt;</td>
<td>500&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1000&lt;sup&gt;c&lt;/sup&gt;</td>
<td>500&lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

<sup>a</sup> Expressed in μg/l.
<sup>b</sup> Temporary MRLs, pending the receipt of further information (see below).
<sup>c</sup> No MRLs could be recommended for eggs because no additional data were available on residues in eggs.

The following information is required for evaluation in 1999:
1. Validation of the HPLC assay to measure residues of streptomycin.
2. Studies to determine whether the HPLC and antimicrobial assay methods give similar results for residues of both drugs using tissues with incurred residues.

Enrofloxacin

ADI: 0–2 μg per kg of body weight.

Residue definition: The evaluation of residues was postponed due to the late submission of important additional data.

Flumequine

ADI: 0–30 μg per kg of body weight.

Residue definition: Flumequine.

<table>
<thead>
<tr>
<th>Species</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>500</td>
<td>1000</td>
<td>3000</td>
<td>1000</td>
</tr>
<tr>
<td>Pigs</td>
<td>500&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1000&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3000&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1000&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sheep</td>
<td>500&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1000&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3000&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1000&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chickens</td>
<td>500&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1000&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3000&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1000&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Trout</td>
<td>500&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

<sup>a</sup> Temporary MRLs.
<sup>b</sup> Muscle and skin in normal proportions.
The Committee requested that studies be conducted with radiolabelled flumequine in pigs, sheep, chickens and trout to estimate the proportion of the total residues accounted for by the parent drug. The results of these studies are required for evaluation in 2000.

**Gentamicin**

**ADI:** 0–4μg per kg of body weight (temporary ADI; established at the forty-third meeting of the Committee (WHO Technical Report Series, No. 855, 1995)).

**Residue definition:** Gentamicin (MRLs 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>100b</td>
</tr>
<tr>
<td>Pigs</td>
<td>100b</td>
</tr>
</tbody>
</table>

* Expressed as μg/l.

**Spiramycin**

**ADI:** 0–50μg 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 (except for cows' milk, all MRLs were recommended at the forty-seventh meeting of the Committee (WHO Technical Report Series, No. 876, 1998)).

Spiramycin equivalents for pigs (MRLs were recommended at the forty-seventh meeting of the Committee (WHO Technical Report Series, No. 876, 1998)).

<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>200b</td>
</tr>
<tr>
<td>Pigs</td>
<td>200c</td>
</tr>
<tr>
<td>Chickens</td>
<td>200b</td>
</tr>
</tbody>
</table>

* Expressed as μg/l.
* Expressed as the sum of the concentrations of spiramycin and neospiramycin.
* Expressed as spiramycin equivalents.
Glucocorticosteroid

*Dexamethasone*

ADI:  
0–0.015 µg per kg of body weight (established at the forty-second meeting of the Committee (WHO Technical Report Series, No. 851, 1995)).

Residue definition: Dexamethasone (temporary MRLs recommended at the forty-second and forty-third meetings of the Committee (WHO Technical Report Series, No. 851, 1995; WHO Technical Report Series, No. 855, 1995) were not extended due to the absence of data on a method for use in regulatory monitoring, which was requested at the forty-second meeting).

Insecticides

*Cyfluthrin*

ADI:  
0–20 µg per kg of body weight.

Residue definition: Cyfluthrin.

<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>20</td>
</tr>
</tbody>
</table>

*Expressed in µg/l.

*Fluazuron*

ADI:  
0–40 µg per kg of body weight.

Residue definition: Fluazuron.

<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</td>
</tr>
</tbody>
</table>
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---|---|---
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